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Finding Genes Underlying Schizophrenia Retinoid and Thyroid Hormone Hypotheses

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Finding Genes Underlying Schizophrenia: Retinoid and Thyroid Hormone Hypotheses

Estudo das causas moleculares e genéticas da esquizofrenia: hipótese dos retinoides e das hormonas tiroideias

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Obviously these acknowledgments are far from complete, which I hope to amend through more personal channels.

Summary

Evidence from multiple lines of research supports the hypothesis that schizophrenia arises from interactions between genetic and environmental factors during critical early periods of neuronal development.

Abnormal levels of thyroid hormones or retinoids during fetal development have been suggested to contribute to the neurodevelopmental deviations found in schizophrenic patients. In fact, thyroid hormones and retinoids could constitute a functional link between genetic and environmental risk factors in schizophrenia. This is an attractive hypothesis capable of explaining several epidemiological features of the disease.

Thyroid hormones and retinoids are involved in the regulation of central nervous system function probably through their capacity to modulate the expression of several genes. To investigate if genes involved in the metabolism of thyroid hormones and retinoids account for an increase in schizophrenia susceptibility, we performed association studies in four promising candidate genes, using three independent samples. The proteins encoded by these four genes are involved in molecular pathways implicated in schizophrenia.

Human Nur-related receptor 1 (NR4A2) is an orphan nuclear receptor that regulates transcription by heterodimerization with retinoid nuclear receptors. NR4A2 is essential for the formation of the dopaminergic system, the neurotransmitter pathway targeted by most anti-psychotic drugs used in the treatment of schizophrenia. We did not find, in our samples, any of the *NR4A2* mutations previously described, further confirming that they are rare.

The Lipocalin-type of prostaglandin D2 synthase (PTGDS) transports thyroid hormones and retinoids, and the expression of the gene is regulated by both modulators. In addition, it is involved in lipid metabolism, which is altered

in schizophrenia. We found four previously described polymorphisms, none of which was associated with schizophrenia.

Transthyretin (TTR) is a transporter of both thyroid hormones and retinoids, and has been implicated in behavior. Neither a novel polymorphism, that we describe for the first time, nor another, previously identified, were associated to the disease. Similarly, serum TTR levels did not differ between patients and mentally healthy individuals.

Neurogranin (NRGN) is a neurospecific protein whose expression is regulated by retinoids and thyroid hormones. Interestingly, NRGN is involved in glutamatergic transmission, known to be abnormal in schizophrenia. We report the association of a DNA variant near the thyroid hormone response element in the *NRGN* gene with schizophrenia in males. The gender specificity of this association is of particular interest since significant symptomatic differences in disease manifestation exist between sexes, such as earlier onset and more severe manifestation in male patients. *NRGN* joins several other genes already implicated in schizophrenia that are likewise involved in the glutamatergic pathway, such as *dysbindin* and *neuregulin*. Thus, our finding introduces an additional player into the glutamatergic hypothesis of schizophrenia.

In a preliminary study, we evaluated thyroid hormone function in schizophrenic patients, which showed reduced levels of free triiodothyronine in males when compared with healthy individuals. Whether this alteration is related to the manifestation of symptoms still needs to be investigated.

Together our data support the involvement of thyroid hormones in schizophrenia, which should be further explored in expression studies.

Resumo

Vários estudos sugerem que a esquizofrenia resulta da interacção entre factores ambientais e genéticos particularmente em períodos críticos do desenvolvimento do sistema nervoso central. Os factores ambientais podem condicionar o desenvolvimento do sistema nervoso central, nomeadamente por influenciarem a expressão de vários genes. Tal é o caso, directa ou indirectamente, da vitamina A e das hormonas tiroideias. Desta forma, estas moléculas podem estabelecer uma ponte entre genes e ambiente em esquizofrenia. Esta hipótese é particularmente atractiva por ser capaz de explicar várias características da esquizofrenia, tais como a sua distribuição epidemiológica. Com o intuito de testar se genes envolvidos no metabolismo quer dos retinóides quer das hormonas tiroideias contribuem para um aumento da susceptibilidade à esquizofrenia, efectuámos estudos de associação em três amostras independentes de doentes. Os genes escolhidos codificam proteínas que fazem parte de cascatas moleculares potencialmente relacionadas com a esquizofrenia.

O NR4A2 é um receptor órfão que, em parceria com receptores do ácido retinóico, regula a transcrição de vários genes. Sabe-se que o NR4A2 é essencial na formação do sistema dopaminérgico, que é a via de neurotransmissores alvo da maioria dos fármacos antipsicóticos. Nas amostras que estudámos, não foi detectada nenhuma das mutações préviamente descritas no gene da *NR4A2*, o que confirma serem mutações extremamente raras.

A expressão do gene que codifica a PTGDS, uma proteína que transporta tanto retinóides como hormonas tiroideias, é regulada por ambos os ligandos. Para além disso, sendo responsável pela produção de prostanóides, está envolvido no metabolismo lipídico que se sabe estar alterado em doentes com

esquizofrenia. A pesquisa de mutações revelou apenas quatro polimorfismos já descritos na literatura, não estando nenhum deles associado com a doença.

Os níveis de TTR, principal transportadora tanto de retinóides como de hormonas tiroideias, têm sido descritos como estando diminuídos em doentes neurológicos e psiquiátricos, e a abolição da expressão do gene como tendo consequências no comportamento. Neste trabalho pesquisámos variantes na *TTR* tendo identificado um novo polimorfismo. Nem este, nem um outro préviamente descrito, se encontram no entanto associados com a esquizofrenia. De igual modo, os níveis séricos de TTR são idênticos entre os doentes e os indivíduos mentalmente saudáveis.

Estudámos, ainda, o gene que codifica a *neurogranina* (*NRGN*) cuja expressão é regulada tanto por retinóides como por hormonas tiroideias. A neurogranina é uma proteína específica dos neurónios e está envolvida na transmissão glutamatérgica que se sabe estar alterada em doentes esquizofrénicos. Encontrámos associação de um polimorfismo que se encontra próximo do elemento de resposta às hormonas tiroideias, em homens com esquizofrenia. A especificidade desta associação em homens é particularmente interessante, uma vez que estão descritas diferenças na idade de início, gravidade da doença e resposta ao tratamento entre homens e mulheres; sendo que nos homens há um início mais precoce e uma forma mais severa da manifestação dos sintomas. Assim sendo, descrevemos, pela primeira vez, a *NRGN* como mais um gene que confere aumento da susceptibilidade para a esquizofrenia. De realçar que a NRGN se encontra tanto a montante como a jusante de vias de sinalização préviamente sugeridas como comprometidas na doença, pelo que pode constituir um ponto de charneira na regulação glutamatérgica.

Finalmente, a medição dos níveis séricos de hormonas tiroideias num estudo piloto indica existirem níveis diminuídos de triiodotironina livre nos doentes do sexo masculino, quando comparados com indivíduos saudáveis. A possibilidade desta alteração estar relacionada com a manifestação da doença necessita ainda de ser investigada.

Em conjunto, os nossos resultados apoiam a hipótese do envolvimento das hormonas tiroideias na esquizofrenia e abrem perspectivas para a pertinência de estudos detalhados ao nível da expressão de genes.

Contents

Abbreviations

ADH	alcohol dehydrogenase
ALB	albumin
ALDH	aldehyde dehydrogenase
CaM	calmodulin
CI	confidence intervals
CNS	central nervous system
COMT	catechol-O-methyltransferase
CRABP	cellular retinoic acid binding protein
CRBP	cellular retinol binding protein
CSF	cerebrospinal fluid
CT	computed tomography
CYP26	cytochrome P450
DAOA	D-amino acid oxidase activator
DIGS	Diagnostic Interview for Genetic Studies
DIO	deiodinase
DSM	Diagnostic and Statistical Manual for mental disorders
DTNBP1	dysbindin
DZ	dizygotic
EGR1	early growth response 1
FT_3	free T_3
FT_4	free T_4
HWE	Hardy-Weinberg Equilibrium
ICD	International Classification of Diseases
LD	linkage disequilibrium
LDL	low-density lipoprotein

LPL	lipoprotein lipase
LRAT	lecithin retinol acyltransferase
LSD	lysergic acid diethylamide
MAP2K4	mitogen-activated protein kinase kinase 4
MRI	magnetic resonance imaging
MZ	monozygotic
NMDA	N-methyl-D-aspartate
NR1	NMDA receptor subunit 1
NR2A	NMDA receptor subunit 2A
NR4A2	Nur-related receptor 1
NRG1	neuregulin 1
NRGN	Neurogranin
Nurr1	the mouse homologue to human NR4A2
OPCRIT	Operational Checklist for Psychotic Disorders
OR	Odds Ratios
PCP	phencyclidine
PDT	pedigree disequilibrium test
PGD2	prostaglandin D2
PKC	protein kinase C
PPAR	peroxisome proliferator-activated receptor
PPP3CC	calcineurin gamma subunit
PRODH	proline dehydrogenase
PTGDS	lipocalin type of prostaglandin D2 synthase
RA	retinoic acid
RAL	retinal
RAR	retinoic acid receptor
RARE	RA response element
RBP	retinol-binding protein
RFLP	restriction fragment length polymorphism
RGS4	regulator of G-protein signaling 4
RoDH	retinol dehydrogenase
ROH	retinol
RORA	RAR-related orphan receptor A

RT-PCR	real-time polymerase chain reaction
RXR	retinoid X receptor
SDR	short-chain dehydrogenase/reductase
SNP	single nucleotide polymorphism
SSCP	Single Strand Conformational Polymorphism
T_3	triiodothyronine
T_4	thyroxine
TBG	thyroxine-binding globulin
TDT	Transmission disequilibrium test
TH	Thyroid hormone
TNFRSF6	tumor necrosis factor receptor superfamily, member 6
TR	thyroid hormone receptor
TRAP	TH-associated protein
TRAR4	trace amine receptor 4
TRE	TH response elements
TRH	thyrotrophin-releasing hormone
TSH	thyroid-stimulating hormone
TT_3	total T_3
TT_4	total T_4
TTR	transthyretin
VCFS	velo-cardio-facial syndrome

1

General introduction

Schizophrenia is a severe and disabling mental illness. It is characterized by disturbances in brain function, leading to a maladjustment to reality. Patients present a wide range of symptoms and signs indicative of alteration in cognitive, emotional, motivational, perceptual, and motor functions [210]. So far, no cure exists, which makes schizophrenia a chronic disorder. The currently available medication is not effective in all patients, and often provides only partial alleviation of disease symptoms at the cost of significant side effects for many patients [112].

Both genetic and environmental risk factors are believed to participate in the etiology of the disease. Establishing the genetic causes underlying schizophrenia would offer the opportunity not only for using a molecular diagnosis but also for the development of more effective treatments. The work presented in this thesis contributes to the progress in schizophrenia research by investigating whether two classes of modulators of gene expression, namely thyroid hormones and retinoids, are involved in the etiology of this disorder.

To introduce the reader to the main body of the material presented in Chapters 3 through 7, we first embark on a review of the schizophrenia disorder, including its prevalence, its diagnosis and known facts about its physiological, genetic, and environmental background (Section 1.1). Nowadays, the view that schizophrenia is a neurodevelopmental disorder is commonly accepted. We will discuss this view (Section 1.1.7) and how the thyroid hormone and retinoid hypotheses fit in it (Section 1.2). Finally, we will describe the types of approaches most commonly pursued in the molecular study of the genetic basis of schizophrenia (Section 1.3).

1.1 Schizophrenia

Schizophrenia is a heterogeneous disorder identified on the basis of a pattern of abnormal behavior, characterized by false beliefs and abnormal perceptions. The symptoms presented by schizophrenic patients can be divided into two main types. The “positive symptoms” refer to normal functions that are distorted or exaggerated. These include hallucinations or abnormalities

in perception; delusions or abnormalities in inferential thinking; disorganized speech or abnormalities in language; disorganized behavior or abnormalities in behavioral monitoring and control. The “negative symptoms” are those that represent diminution or loss of normal mental functions. These include alogia or a decrease in the fluency of ideas and language with consequent difficulty in maintaining a continuous conversation; affective blunting or difficulty in expressing emotions; avolition or extreme apathy with lack of initiative, drive, and energy to pursue goal-directed activities; anhedonia or lack of pleasure and interest in life; attention impairment or a decrease in the ability to focus attention (reviewed in [13]). Note that none of these symptoms are specific to schizophrenia, nor present in all patients.

In many schizophrenic patients cognitive impairments are also observed. Patients with schizophrenia generally perform poorly on psychological tests of attention, memory, executive functions, and information processing. Interestingly, these impairments are also observed in individuals with high risk of developing schizophrenia, and have been proposed as predictive or as early signs of the illness [96]. Although more severe in patients, cognitive deficits are also present in their unaffected siblings [48], which suggests that cognitive deficits are heritable. It has been noted that these cognitive deficits are usually nonprogressive and relatively stable over time [123, 143].

It has been proposed that patients with predominantly positive symptoms more often have acute onset, good prognosis, and favorable response to medication. In contrast, patients with predominantly negative symptoms more often present a chronic course, poor prognosis, inadequate response to medication, and evidence of cognitive impairment [37].

1.1.1 Prevalence

Although the clinical course of schizophrenia varies considerably between populations, the median lifetime prevalence of 0.7-0.8% [324] shows surprisingly little variation across populations that vary widely in climatic and social environment, and that have been genetically separated for many thousands of years [171]. This fact has been interpreted as a result of a schizophrenia

genesis early during the evolution of modern *Homo sapiens* combined with a possible evolutionary advantage associated with the trait [30]. This could explain the persistence of schizophrenia despite the decrease in procreation observed in patients [126].

Typically, the age of onset of schizophrenia ranges from mid- to late adolescence through early adulthood, and usually it manifests itself 3 to 5 years earlier in males [171, 98]. It is widely accepted that in younger age groups, the risk of developing schizophrenia is higher for males while beyond age 40 the risk is higher for females [10]. In addition, a higher incidence of schizophrenia in males has been reported in developed countries [10]. However, the existence of sex differences in life time morbid risk is not consensual. In fact, it has been proposed that the reported gender difference regarding prevalence could arise from higher severity of the illness in males, which would make it easier to recognize [208].

In any case, symptomatic sex differences have become obvious in recent studies. Male sex is associated to an earlier onset and a more severe course of the illness, with a higher proportion of male patients being refractory to the treatment and with aggression and violent behaviors being also more common in males. Males also show more negative symptoms and cognitive deficits. Female patients present more affective symptoms, auditory hallucinations, and delusions, with faster and greater response to medication but with increased level of side effects [208]. Whether brain pharmacological effects of reproductive hormones are associated with sex differences found in patients is still unclear. However, the existence of a link between the later age of schizophrenia onset among woman and their higher estrogen secretion has been hypothesized [305]. Higher cognitive abilities have been reported in female patients with higher levels of estrogen [150]. Confirming this observation, it was shown that women to whom estrogen has been administered present a better clinical response to treatment [192]. In addition, lower estrogen levels were reported in psychotic patients in comparison to healthy subjects [158].

Several models have been proposed to explain the observed symptomatic differences between male and female schizophrenic patients, but to date none is regarded as conclusive [208].

1.1.2 Diagnostic methods

The clinical definition of schizophrenia is particularly difficult because of interindividual variation, with the diagnostic decision being made by drawing an artificial line in a continuous behavior scale ranging from normal to schizophrenic [126]. The diagnosis of schizophrenia is mostly done in accordance with two standardized classification systems that have been extensively tested and revised to improve their reliability and validity: the Diagnostic and Statistical Manual for mental disorders, currently in its fourth edition (DSM-IV) [12]; and the International Classification of Diseases, now in its tenth edition (ICD-10) [410]. The two classification systems take a purely descriptive approach and define schizophrenia on the basis of presenting clinical signs and symptoms. The systems do agree and essentially differ only in terminology [126]. These are comprehensive and time-consuming diagnostic tools. An alternative is the Operational Checklist for Psychotic Disorders (OPCRIT), a classification system for mental illnesses which provides a convenient, reliable, rapid, and valid approach to diagnostic assessment [237, 21].

The Diagnostic Interview for Genetic Studies (DIGS) is a commonly used semi-structured interview that combines information from many different diagnostic tools, including those mentioned above [283]. The purpose of the DIGS is to provide researchers in psychiatric genetics with the broadest possible dataset and to ensure maximum compatibility with datasets obtained through other instruments. These features of DIGS make it particularly valuable for the precise and reliable definition of populations for genetic studies. The DIGS provides a rich collection of details about the phenomenology of schizophrenia and mood disorders, more so than many other diagnostic instruments. It brings together not only items found in several other systems but also includes further items regarding mixed states of mania and depression, rapid cycling, suicidality, co-morbidity, and course of illness. Furthermore, the DIGS provides comprehensive assessment of substance consumption, abuse, and dependence, which is of prime importance to prevent false positives. Such extensive phenomenologic detail may allow the construction of quantitative phenotypes or the definition of new biologically based categories [283].

The original DIGS, which uses the English language, has been translated to various other languages, including Portuguese [20]. The Portuguese version has been used to assess individuals in the Portuguese and Brazilian samples used in our studies. The translation to Portuguese has been meticulously performed to preclude distortions, also on the conceptual level. An independent reverse translation has been compared with the original to verify close correspondence.

The reliability of the DIGS has been assessed through various test-retest and inter-rater experiments, and has been found to be high or excellent for the schizophrenia disorder [283, 299]. The inter-rater reliability of the Portuguese version was also studied, and found to be excellent [20].

As mentioned above, schizophrenia does not constitute a homogeneous phenotype. Its standard diagnosis may include a variety of overlapping conditions with different symptoms, courses, outcomes, responses to treatment, and etiologies. In fact, given the complexity of brain functions, classification of schizophrenia based on observed behavior might not be optimal for the genetic dissection of the disease. One increasingly popular strategy is trying to understand the genetics of schizophrenia by understanding the genetics and gene linkage of specific trait markers, known as endophenotypes. Endophenotypes include neurophysiological, biochemical, endocrinological, neuroanatomical, and cognitive measurements more consistently associated with a genetic trait than the clinical symptoms. Such traits should by definition be heritable, more common in affected individuals than in the general population, and also expressed at higher rate in unaffected biological relatives, marking them as carriers of the predisposing alleles [106]. For schizophrenia, some success has been achieved in establishing linkage between specific chromosomal *loci* and specific endophenotypes.

A substantial body of evidence indicates that schizophrenia patients present ocular motor abnormalities. In particular, the normal smooth pursuit of a moving target with the eyes is fragmented, resulting in abrupt, darting eye movements (saccade). Such eye-tracking abnormalities are present both in patients suffering from schizophrenia (50 to 80%) and in their first-degree relatives (40%) in a higher percentage than in the general population (8%). This ob-

servation suggests that it could be used as a phenotypic marker for finding schizophrenia susceptibility genes [152]. A positive linkage to this trait was reported on chromosome 6p [17].

The P50 auditory evoked response is an information processing paradigm involving exposure to two paired stimuli. An initial stimulus induces an excitatory reaction in the brain that also activates inhibitory mechanisms, dampening the excitatory response to a subsequent identical stimulus. Inhibition of the P50 response to the second stimulus is present in 90% of the general population, but in only 50% of schizophrenic patients [107]. Family members of schizophrenic patients also present decreased P50 inhibition which suggests that it could function as trait marker, and its use in linkage analyses may facilitate the identification of genetic liability to schizophrenia [6]. In fact, positive linkage to this trait was reported on chromosome 15q14 [107].

Other trait markers have also been suggested as endophenotypes [127]. In particular, brain imaging studies may reveal novel anatomical and functional disease-specific features relevant for the diagnosis of schizophrenia.

1.1.3 Affected brain structures

Several neuroimaging techniques, like computed tomography (CT) and magnetic resonance imaging (MRI), along with conventional postmortem studies, have been used to examine the anatomical changes present in the brains of schizophrenic patients.

Early histopathological approaches to identifying postmortem abnormalities in brains of schizophrenic patients were too crude to consistently detect the small and subtle abnormalities believed to be involved in schizophrenia. Nevertheless, they established the absence of massive cell loss or glyosis [141]. More recent histopathological examinations have reported an increase in cell-packing density without a change in neuronal number, indicating a decrease in neuropil density and a reduction in dendritic spine density in the pyramidal neurons of the prefrontal cortex [118, 336].

The development of neuroimaging techniques, first CT in the late 1970s and shortly after MRI, produced a revolution in the field since it enabled the

evaluation of brain structural alterations without the limitations of postmortem brain studies. Although both techniques allow detection of neuromorphological alterations as well as assessment of cerebral volumes, MRI became the standard tool for assessing changes in brain structure since it provides greater spatial resolution and high contrast resolution for neuroanatomical structures and grey-white matter distinction than the CT technique. In addition, MRI allows greater flexibility for adapting acquisition parameters to the particular tissues and regions under study. MRI has yet another advantage over CT. While CT involves exposure to ionizing radiation, during an MRI procedure the patient is only submitted to a magnetic field, to which no known biological risks are associated.

Structural analysis revealed that ventricular enlargement is the most consistently found anatomical alteration in schizophrenic brains. Over 75% of CT studies and 80% of the MRI studies in schizophrenia revealed enlarged lateral ventricles [340]. However, it should be noted that such alteration is not specific to the schizophrenia pathology and can also be found in other disorders, including Alzheimer's disease and hydrocephalus [8].

Some MRI studies also show a reduction in whole-brain volume, but this reduction is relatively small [392]. If we assume that these subtle whole-brain differences do not reflect uniform affection of all neuroanatomical regions, we may expect that examination of specific areas might show more substantial relative decreases in volume. Volumetric MRI scans have detected regional brain changes with specific gray matter volume reduction particularly in the superior temporal gyrus and in medial temporal structures, such as the amygdala and the hippocampus [202, 340].

Valuable information stems from studies of monozygotic (MZ) twins discordant for schizophrenia. An MRI study of discordant MZ twins indicated that the schizophrenic twin can be characterized by brain ventricular enlargement, and reduction of hippocampus and temporal lobe when compared with the non-schizophrenic twin [355]. This, together with the observation that unaffected relatives have smaller ventricles than schizophrenic patients, but larger than control subjects from families without schizophrenia [203, 339], supports that ventricular enlargement is associated with the disease and that it is caused by

the effect of environmental factors in genetically susceptible individuals. Additionally, a longitudinal study of adolescents at high risk of developing schizophrenia showed that brain ventricular enlargement correlated positively with genetic risk of schizophrenia in adulthood [182].

In summary, histopathological and neuroimaging studies demonstrate subtle structural brain changes that provide clues regarding the pathophysiology of schizophrenia.

1.1.4 Pharmacological aspects

The first attempts to understand the biochemical basis underlying schizophrenia were based on the mechanisms of action of the drugs capable of alleviating disease symptoms. These drugs, known as antipsychotics or neuroleptics, are usually divided in two classes: typical and atypical.

Typical neuroleptics were introduced in the early 1950s. Some of the typical neuroleptics frequently used are: haloperidol (Ortho-McNeil Pharmaceutical, Raritan, NJ, USA), chlorpromazine (Smith, Kline and French, Ltd., Philadelphia, PA, USA), promazine (Wyeth Pharmaceuticals, Philadelphia, PA, USA), and thioridazine (Sandoz Pharmaceuticals, East Hanover, NJ, USA). Based on the mechanism of action of these drugs, schizophrenia was attributed to hyperactivity of dopaminergic transmission [84]. The antipsychotic action of these first generation drugs correlates with their ability to block dopamine receptors, particularly the D2 subtype of dopamine receptors [335]. Morover, dopamine agonists like amphetamines and cocaine, which act by promoting the release of dopamine, can induce psychosis in healthy individuals as well as aggravate schizophrenic symptoms in patients [51]. Direct validation of dopamine dysregulation in schizophrenia has come from brain imaging studies in schizophrenic patients, which showed higher release of dopamine at the synaptic junction in response to amphetamine stimulation than in non-schizophrenic controls [4, 38].

With time, it became apparent that these classical drugs are not effective in the treatment of all patients and, when effective, mainly reduce positive symptoms [206]. Furthermore, they frequently induce neurological side

effects. While the antipsychotic effects probably result from blockage of dopamine receptors in limbic areas, such as the nucleus accumbens and prefrontal cortex of the brain, the Parkinsonean side-effects provoked by these neuroleptics is caused by D2 receptor blockage in the caudate putamen region [206].

Doubts about the requirement of direct blockage of D2 receptors for antipsychotic drug effectiveness, were raised by the introduction of atypical neuroleptics. These include clozapine (Novartis, Switzerland), olanzapine (Lilly, Indiana, USA), quetiapine (Astra-Zeneca, Sweden) and risperidone (Janssen, Belgium). These neuroleptics were found to be more effective than classical drugs. They do not only reduce positive but also negative symptoms, display a lower incidence of side effects, and clozapine in particular can cause substantial improvement in patients who fail to respond to other neuroleptics [43, 206]. Besides influencing the dopaminergic pathway, these novel drugs also have affinity for other types of neurotransmitter receptors, such as those for serotonin and glutamate.

Serotonin may play a role in the schizophrenia pathophysiology. Supporting this hypothesis is the observation that atypical drugs present greater potency then typical ones to block 5-HT2 subtype of serotonin receptor [241], and that structural similarities exist between serotonin and LSD (lysergic acid diethylamide), a potent hallucinogen. It has been shown that serotonin receptors and its uptake sites are altered in the limbic system of individuals suffering from schizophrenia [174].

The glutamatergic system has also received considerable attention. In particular, a hypofunction of N-methyl-D-aspartate (NMDA) glutamate receptor has been proposed [368]. This hypothesis has arisen from the observation that NMDA receptor antagonists, such as phencyclidine (PCP), ketamine, and MK-801, mimic schizophrenia in healthy individuals even more faithfully than amphetamine, since their administration results not only in positive symptoms but also in negative symptoms and cognitive impairments [361]. Furthermore, they are capable of exacerbating symptoms in schizophrenic patients [193, 206]. These NMDA antagonists act by blocking the binding of glutamate to the NMDA receptor. Notably, treatment with clozapine, but not with the typical neuroleptic halopiredol, attenuates clinical symptoms produced by

ketamine [222], possibly by increasing the expression of NMDA-receptor which facilitates glutamatergic transmission [121]. Additionally, transgenic mice with reduced expression of NMDA receptor subunit 1 (NR1) or lacking the NMDA receptor $\varepsilon 1$ subunit orthologous to the human NMDA receptor subunit 2A (NR2A) exhibit the same behavioral abnormalities as induced by NMDA antagonists in animal models of schizophrenia [259, 256]. Interestingly, kynurenic acid, an endogenous antagonist of NMDA receptors, has been reported to be increased in the hippocampus in postmortem studies of schizophrenics [334].

Unlike other neurotransmitter receptors, the NMDA receptor requires activation by two types of molecules. Besides the glutamate recognition site, the NMDA receptor also presents a distinct site for binding glycine or the D-isomer of serine depending on the brain area [272]. The glutamate hypothesis of schizophrenia has found strong support in studies establishing that agents acting at the glycine modulator site of the NMDA receptor cause symptomatic improvements in patients [147, 369]. Note that administration of direct agonists at the NMDA receptor has not been tried because of the risk that excessive glutamate stimulation may cause excitotoxic damage to neurons [201].

It is important to point out that the three alternative hypotheses presented above are not necessarily mutually exclusive since the various neurotransmission systems are known to interact [284]. In fact, recent theories suggest that glutamate receptors play a central role in the aberrant neurological processing found in schizophrenic patients [257], and that dopaminergic and serotonergic changes may be secondary to the altered glutamatergic transmission [7, 198].

Although these theories attempt to describe the occurring dysfunction, they provide no explanation of the etiological events underlying schizophrenia.

1.1.5 Genetic epidemiology

The classical genetic epidemiological approach of family, adoptee, and twin studies has been remarkably consistent in establishing a significant role for genetic inheritance in the etiology of schizophrenia [238].

Evidence from family studies clearly demonstrates that schizophrenia aggregates in families, and that the risk of developing schizophrenia is directly

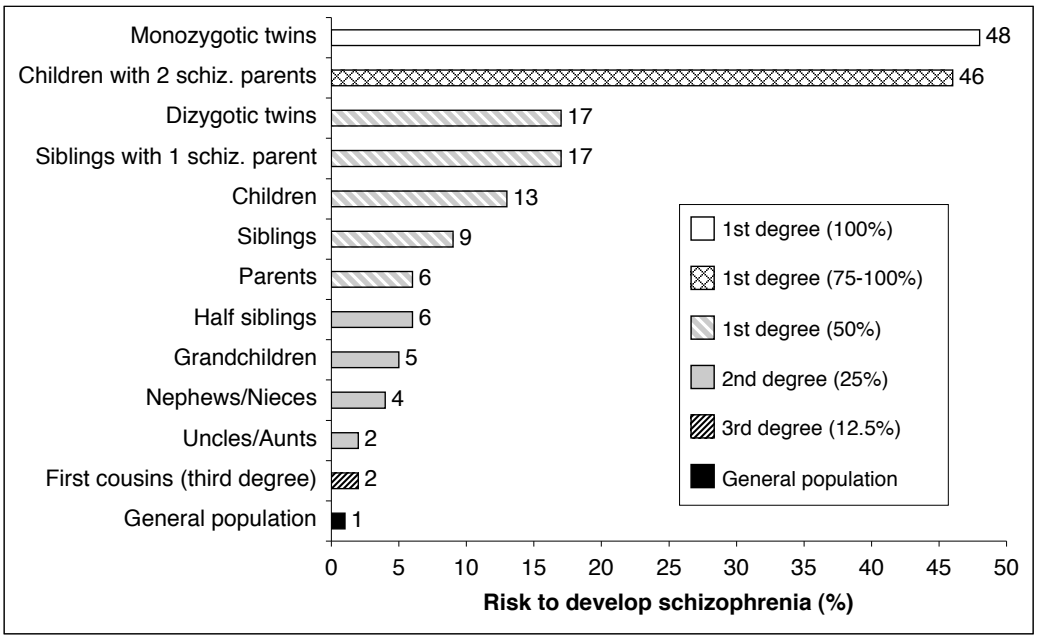

Figure 1.1: Each bar represents the risk to develop schizophrenia, in percentage. The degree of genetic relationship to the affected individual is represented by the bar pattern. The data are based on the review of studies collected by Gottesman [126].

linked to the degree of genetic relationship to an affected individual [262]. Figure 1.1 summarizes the evidence. Children and siblings of individuals with schizophrenia present a risk of developing the disorder that is roughly 10 times higher than the normal population. For second and third degree relatives, the risk is also increased, in smaller, but consistent degree. Moreover, the risk increases with the number of affected relatives. For example, the risk is 13% if only one parent is ill, and 46% if both parents are affected. Schizophrenia often coexists in families with other psychotic disorders [181].

Adoption studies indicate that children of individuals with schizophrenia, when adopted, still present a higher risk of developing schizophrenia, as expected for first-degree relatives [167, 363]. In biological relatives of schizophrenic adoptees, disease incidence is higher, and in the adoptive families of both affected and control adoptees, schizophrenia rates are low and similar [183]. Thus, the increased risk found in families is related to genetic factors and cannot be explained by family environment or by the stress of living with affected individuals.

Twin studies have provided the most significant evidence for the presence of a genetic component. The methodology of twin studies is based on the observation that while monozygotic (MZ) twins have identical genotype, dizygotic (DZ) twins share, on average, 50 percent of their genes. Therefore, if genes play a major role in disease susceptibility, the concordance rates (extent to which both members of a twin express the trait) would be considerably higher in MZ than in DZ twins. In fact, it has been consistently shown that schizophrenia concordance rates in MZ twins are approximately 50%, which is higher than the 17% concordance rates found in DZ twins [50], providing conclusive evidence of a genetic effect. The similar risk present in the offspring of discordant MZ twins [126] provides evidence that the cause of discordance in MZ twins is due to environmental and/or epigenetic factors.

The patterns of schizophrenia risk, observed in family and twin studies, provide clues regarding the mode of transmission of the disease. The fact that the risk for schizophrenia is higher in close relatives of affected individuals, but decreases rapidly in more distant relatives, implies that a single-locus transmission model of the disease must be rejected [290]. In fact, the involvement of multiple genes is supported by linkage studies that have identified several chromosomal regions as potentially containing risk genes (see Section 1.3).

A possible explanation of the observed risk patterns is provided by a polygenic multifactorial model of transmission, with a threshold effect for disease manifestation [126]. This model assumes a complex etiology involving multiple genes and environmental factors. The effects of each factor combine additively (total liability from several factors is the sum of liabilities of each factor separately), and the trait manifests itself when liability exceeds a certain threshold. Thus, although the schizophrenia diagnosis is dichotomous (present or absent), liability to the disorder is a quantitative measure, normally distributed over the general population. In contrast to a single-gene disorder, in a polygenic disease, possession of a particular susceptibility gene does not mean that an individual will necessarily develop the disease, but just that he/she is at increased risk; in fact, the majority of carriers are not expected to express any clinical phenotype [214]. Only individuals who are above a certain threshold of liability due to a combination of risk factors, will develop the disease. The

incidence of the disorder is greatest among relatives of affected individuals because they are more likely to aggregate several of these risk factors and thus on average have a bigger chance to be above the threshold, i.e. they have a higher liability to develop the disorder than the general population.

Apart from the polygenic multifactorial threshold model, the observed patterns of risk of schizophrenia are also compatible with a model of multiple *loci* with epistatic interaction. In such an epistatic model, the rapid increase of risk with degree of genetic relatedness is explained by non-additive combination of gene effects, where interactions between genes confer a total liability greater than the sum of their individual effects [311].

In conclusion, in the majority of cases, schizophrenia probably results from a combination of many risk genes, each of small effect, which, as the MZ twin discordance rate of 52% imply, must be substantially modulated by environmental factors.

1.1.6 Environment

Epidemiological studies have identified a number of environmental factors, such as maternal malnutrition, prenatal infections, and obstetric complications, associated with increased rates of schizophrenia in at least some patient groups [47, 160]. These early events can explain a significant portion of the variance, although they do not have as much predictive power as the genetic factors. In fact, most of the individuals that experience these sorts of adverse environmental events do not develop schizophrenia [154]. Most likely, they need to be combined with additional susceptibility factors to lead to illness. The environmental stressors implicated can be subdivided according to the time in which they exert their effect: in the prenatal period, the perinatal period, or during childhood and early adolescence.

Prenatal period

Two independent studies have reported an association between prenatal exposure to severe malnutrition and the offspring's risk for schizophrenia in adult-

hood, especially if the exposure is in the first trimester of gestation [350, 358]. Although the Dutch hunger winter of 1944-1945 and the Chinese famine of 1959-1961 represent different circumstances of famine, culture, and ethnicity, the findings are remarkably consistent, with the two studies reporting a twofold increase in risk for schizophrenia in both male and female offspring prenatally exposed to famine. Interestingly, it seems that the early pregnancy exposure to famine only increases the risk for schizophrenia and not for other psychiatric disorders [358].

A series of studies on influenza epidemics have reported, though not consistently, an increased risk of developing schizophrenia in the offspring exposed during gestation, with some studies indicating that the increase is especially noticeable when the infection occurs during the second trimester of gestation [236]. Even when considering positive results, the observation is that influenza can account for only a small minority of cases. More recently, elevated serum cytokine levels in mothers prior to delivery of individuals who later develop schizophrenia have again implicated influenza, rubella, and respiratory infections in the schizophrenia etiology [41, 42, 45]. In addition, recent reports provide further indications for a role of the immune system in the schizophrenia etiology [245, 243]. However, it should be noted that influenza exposure *in utero* has been reported to also increase the risk to other psychiatric disorders [219, 244, 14].

Experimental studies in animals have provided support for the influence of viral infection in schizophrenia risk. Infection of pregnant mice with human influenza virus results in offspring that display several “schizophrenia-like” behaviors [341]. Moreover, similar behavioral changes were detected in the offspring of mice who where submitted, during pregnancy, to immune challenges that mimic a viral infection [341]. This provides evidence for the influence of the immune response on brain development.

Perinatal period

Obstetric complications, during pregnancy or at the time of delivery, are reported to be more frequently observed in persons who later develop schizo-

phrenia. A meta-analysis reported that people exposed to obstetric complications have twice the risk to develop schizophrenia with respect to people that did not suffer from obstetric complications [113]. In a large, more recent study, the increased risk for schizophrenia was observed for several obstetric complications, such as preeclampsia, low birth weight, and severe prematurity (gestational age below 33 weeks) [68]. In this study preeclampsia seems to be the stronger risk factor with an increase of risk between 2 to 2.5 times. Interestingly, preeclampsia, which occurs during the third trimester of pregnancy and is characterized by edema, high blood pressure, and the presence of protein in the urine, is associated with a reduction in fetal nutrition [225]. Also, low birth weight, when corrected for gestational age, can be considered a sign of malnutrition. Therefore, the precise factors that relate obstetric complications with schizophrenia, if confirmed, still need to be identified.

Although obstetric complications were consistently associated with schizophrenia their overall effect is small. That is, the presence of obstetric complications has a low predictive value for schizophrenia manifestation and their presence is not sufficient for the illness to appear. Studies of the effect of obstetric complications on brain development of patients, their unaffected relatives, and controls, have provided evidence for the interaction between genetic and environmental factors during neurodevelopment [234, 333]. These studies have shown that obstetric complications have a restricted effect in brain development of control subjects, while in patients they induce a decrease in left hippocampal volume and an increase in lateral ventricular volume. The same alterations found in schizophrenics were also observed in non-affected relatives, though less pronounced. This suggests that obstetric complications may have a greater effect in brain development of individuals that have a genetic susceptibility to schizophrenia compared to those that do not have this predisposition. Finally, note that obstetric complications appear to increase the risk not only for schizophrenia, but also for affective disorder [223].

Childhood and early adolescence

Other environmental risk factors seem to have a more postnatal influence, such as growing up in an urban area, migration or minority status, and the use of drugs, in particular of cannabis.

Recent studies have shown that individuals that grow up in an urban environment present an increased risk of schizophrenia when compared with those growing up in rural areas [287]. However, it is not clear whether this association actually reflects a higher exposure to other factors, such as infection, toxins, or malnutrition, which may be more common in urban populations.

It has been reported that migrants present a higher incidence of schizophrenia, possibly due to chronic exposure to discrimination [163]. In a recent review, the relative risk for developing schizophrenia was indicated to range from 2.7 in first-generation migrants to 4.4 in second-generation migrants [49].

These observations are supported by social isolation studies in animals, which show association between temporal loss of maternal care in critical early periods of rodent development and changes in the dopamine system characterized by enhanced dopamine release after amphetamine administration [135]. These molecular changes induce long lasting alterations in behavior, similar to those found in schizophrenia [92, 162]. In contrast, when similar studies are conducted in post-weaning rats (isolation rearing) the behavior impairment is not permanent [377] and can be prevented if for instance the isolation-reared mice are handled [190]. Altogether, these studies suggest that stress is more disruptive in early stages of brain development. Interestingly, the behavioral alterations only become manifest after “puberty” in rats, and persist thereafter, though both typical and atypical antipsychotics can restore the normal behavior [92]. Additionally, the fact that not all rat strains display behavior alteration when submitted to social isolation, emphasizes the role of genetic background in the effects of pre- and neo-natal manipulations [398, 91]. Note that social isolation during postnatal development has been reported to predispose also to depression and anxiety behaviors in adulthood [144, 399, 131].

The use of psychoactive drugs, in particular cannabis, during adolescence

was found to be associated with an increased risk of psychosis in adulthood [18, 136]. Interestingly, a recent study revealed that individuals with schizophrenic affected relatives were more likely to develop prolonged psychosis after the drug use than subjects who had no family history of schizophrenia [146]. This suggests that the use of dopamine-releasing drugs is acting as a trigger in individuals that are particularly sensitive to dopamine, which by exceeding the threshold results in psychosis.

The influence of these childhood and early adolescence factors seems to fit in the so-called "vulnerability-stress" model of the schizophrenia etiology [421, 381]. This model suggests that predisposed individuals will manifest the disease particularly under conditions of stress which may be either psychosocial or physical. This model does not exclude the presence of strong genetic predisposition in which the illness would be manifest even without these stressors.

The main idea that schizophrenia has its etiology in gene-environment interactions acting during brain development does not specify any particular gene, or environmental factor that can be responsible for the neurodevelopmental impairment. However, data from a variety of sources support that, independently of the specific genetic and environmental causative agents, schizophrenia is a disease with genesis in brain development [304].

1.1.7 Abnormal neurodevelopment

The view of schizophrenia as a neurodevelopmental disorder is based on observations suggesting that the abnormalities found in schizophrenia have an early onset in the life of individuals who later develop the disorder. The neurodevelopmental theory of schizophrenia is based on robust evidence derived from diverse sources (for a review see [225]), and proposes that genetic susceptibility combined with environmental perturbations during critical periods of neural development lead to abnormal neurodevelopmental trajectories. Views differ in the timings and nature of the disturbance(s). Still, based on predictions that many environmental events associated with an increased risk for schizophrenia occur during prenatal and perinatal periods of life, it is likely that

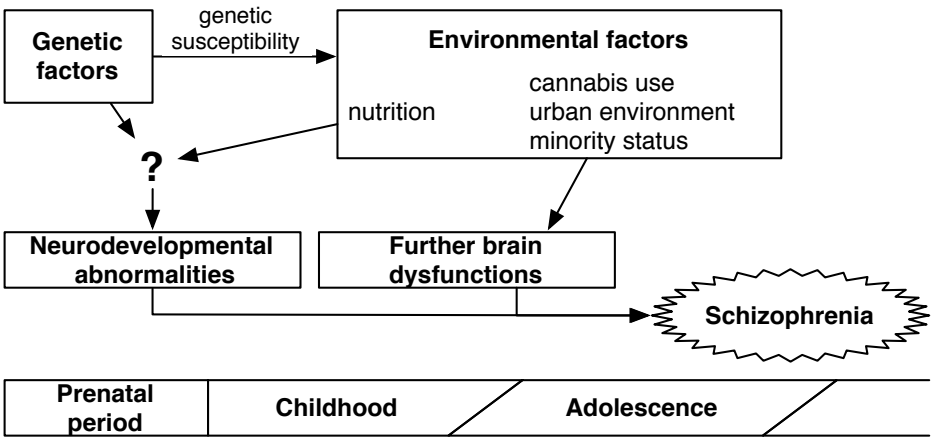

Figure 1.2: According to the neurodevelopmental hypothesis of schizophrenia, genetic factors combine with environmental events during early periods in life to produce neurodevelopmental abnormalities that predispose to schizophrenia. The specific way in which these factors interplay remains to be elucidated. Additional environmental insults later in life, such as during adolescence, may cause neurodevelopmentally impaired individuals to actually develop schizophrenia.

brain development begins to deviate already *in utero* and is probably affected by events around birth, and that environmental stressors such as drugs and an adverse social environment around adolescence may drive neurodevelopmentally impaired individuals over some threshold, inducing the clinical phenotype recognized as schizophrenia [154] (Figure 1.2).

Neuroimaging studies have confirmed that schizophrenia is associated with brain structural abnormalities (see Section 1.1.3). The size reduction in some structures found in brains of schizophrenic patients could have neurodevelopmental or neurodegenerative causation or could result from a combination of the two. The proliferation of glia, the most numerous type of brain cells, to form a glial scar (termed gliosis) has been considered the hallmark of neurodegenerative processes involving cell loss that is not apoptotic. The absence of gliosis in postmortem schizophrenic brains has been interpreted as evidence that the disease is not degenerative in the classical sense and should thus be developmental [397].

Knowing the time when brain changes occur is essential for understanding their significance in the etiology of schizophrenia. A neurodevelopmental model of schizophrenia is supported by the observations that ventricular en-

largement, reduced hippocampal volume, decreased pyramidal cell volume, and lower glial cell density in the medial temporal lobe and hippocampus, are already present at disease onset in first-episode patients [396, 294], which excludes the possibility that they are consequences of medication or disease pathophysiology. Moreover, these alterations have also been reported in unaffected young individuals who have a high genetic risk of developing schizophrenia due to their family history [203], supporting the idea that the brain changes precede the disease onset.

A developmental abnormality in schizophrenia is also supported by retrospective clinical observations that pre-schizophrenic individuals often exhibit minor deviations in motor, cognitive, and social development during childhood and adolescence [96, 289]. In addition, an increased frequency of minor physical anomalies such as curved fingers, low-set ears, and high-arched palate, have been detected in schizophrenic individuals [240]. Such slight deviations in external physical characteristics are indicators of problems of fetal development [235]. Additionally, several genes implicated in the schizophrenia etiology (see Section 1.3) can be linked with neurodevelopment such as: *retinoic acid receptor alpha* (*RAR*α) [309], *myelin-basic protein* [134], *G-protein signaling-4* (*RGS4*) [411], *calcineurin* [129], and *neuregulin* [330].

Brain development is a highly complex biological process. It certainty requires the contribution of several genes and non-genetic factors in a tightly controlled program of events. When a specific factor eliminates, alters or postpones a specific development step it is possible that the normal development of the brain is compromised and that it will predispose for later psychiatric problems. However, while it is easy to accept that an abnormal neurodevelopment could cause childhood disorders like autism, it is harder to understand how it can be involved in the schizophrenia etiology in which the behavioral sequels of these early events remain almost completely dormant until after puberty. Nevertheless, we should keep in mind that human brain development is an ongoing process that continues into early adulthood, when maturation of some central circuits probably leads to the emergence of disease symptoms.

1.2 Retinoids and thyroid hormones

The hypothesis of abnormal neurodevelopment caused by gene-environment interactions acting during brain development provides a framework of thought and experiment, but is not testable as such, since it does not specify any particular gene, environmental factor, or pathway that might be involved. In the sequel, we will explain how the hypothesis can be made more concrete and testable.

Gene-environment interactions occurring during brain development establish the way in which nerve cells are laid down, differentiated, selectively culled by apoptosis and remodelled by expansion and retraction of dendrites and synaptic connections. The complex range of schizophrenic symptoms and outcomes could be produced by the multitude of time points at which the normal brain development can be affected. Several gene-environment interactions could be responsible for alterations in neurodevelopment. Inadequate nutritional or hormonal signaling, which disrupts proper gene transcription, could justify the heterogeneity of clinical presentations and the multiple cellular systems affected, depending on the moment during brain development at which the abnormality takes place.

Proper nutrition is crucial to the correct structural and functional development of the central nervous system (CNS). Severe maternal nutritional deficiency has been related to increased offspring's risk for developing schizophrenia later in life, as explained previously (Section 1.1.6). Nevertheless, it is not known whether the increased predisposition arises from a global nutritional deficiency or from a specific micronutrient deficiency. Reduced supply of nutrients, such as oxygen, iron, glucose, vitamin A, and iodine to the fetus may lead to impaired CNS development and consequently influence the risk to develop schizophrenia. Several hypotheses have been formulated in order to serve as mechanistic models of how micronutrients could contribute to schizophrenia genesis.

Below, we will formulate a hypothesis for the schizophrenia etiology based on abnormalities in retinoid and thyroid hormone metabolism.

1.2.1 Influence of retinoids on brain function and behavior

It has been proposed that dysregulation of retinoid signaling may play an important role in the etiology of schizophrenia [194, 125]. Several lines of evidence support this hypothesis.

First, retinoids (vitamin A, or retinol, and its metabolites) regulate cellular proliferation, differentiation, and apoptosis [348]. A proper regulation of these processes is required for a correct formation of the body axis and for the normal development of several organs, including the brain [269]. It has been reported that when retinoic acid (RA) concentrations deviate from normal values during embryonic development, in either direction, abnormal development occurs [221, 151].

Second, excess or deficiency of retinoids during development can produce several signs of schizophrenia, including thought disorder, mental deficit, enlarged ventricles, and minor physical anomalies [125]. Moreover, the fact that these retinoid-related anomalies are often also observed in relatives of schizophrenic patients, suggests that genetic susceptibility to alterations in retinoid metabolism may be present in schizophrenic families [124].

Third, many genes regulated by retinoids or involved in their metabolism are localised in chromosomal regions which have been implicated in schizophrenia by linkage studies [125].

1.2.2 Retinoid metabolism

Vitamin A is an essential nutrient. It can not be synthesised by the human body, and needs to be acquired through diet. During gestation, the maternal diet must make vitamin A available to the developing fetus, via blood supply.

Figure 1.3 presents a schematic overview of the retinoid metabolism. The main sources of vitamin A are provitamin A carotenoids in fruits and vegetables and retinyl esters from food of animal origin [140]. A crucial role in the maintenance of vitamin A homeostasis is performed by the liver that works as a reservoir of retinol, which is drawn from in times of depletion [275]. Retinol is released into circulation by the liver, bound to retinol-binding protein (RBP).

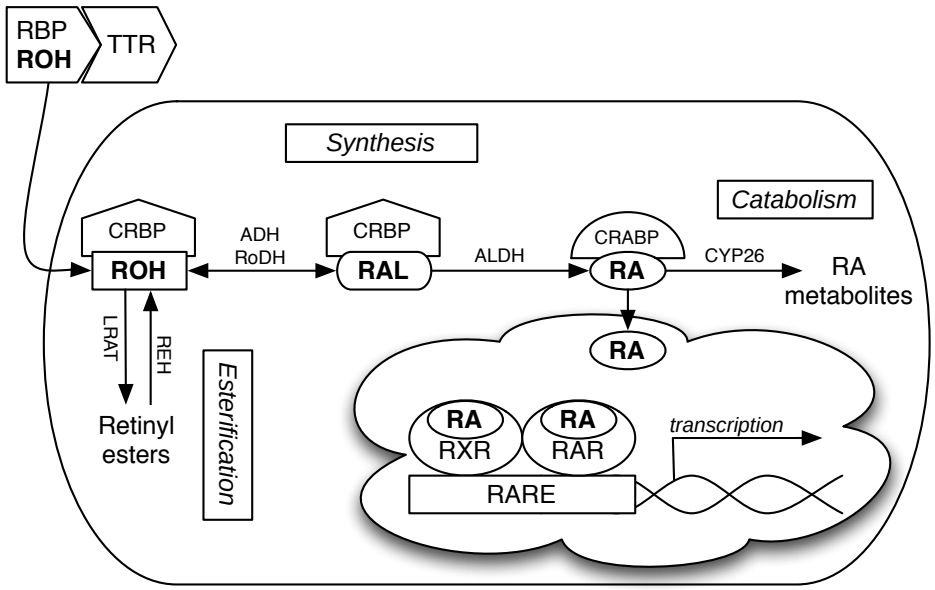

Figure 1.3: Schematic representation of the retinoid metabolism. Retinol (ROH) in the plasma is released from its carrier, retinol-binding protein (RBP) bound to transthyretin (TTR), and enters the cell where it is bound by cellular retinol binding protein (CRBP). The first step of synthesis to retinoic acid (RA) is catalyzed by retinol dehydrogenase (RoDH) or alcohol dehydrogenase (ADH). The synthesized retinal (RAL) remains bound to CRBP and is then oxidized irreversibly to RA, which binds to cellular retinoic acid binding protein (CRABP). RA can either enter the nucleus to bind to its nuclear receptor (RAR or RXR) to activate transcription, or be inactivated by the enzyme CYP26.

The retinol-RBP complex, in turn, is found bound to transthyretin (TTR) [265].

Within cells, the retinoid concentration seems to be buffered by association with soluble proteins: cellular retinol-binding proteins (CRBPs) which bind retinol in the cytoplasm, and cellular RA-binding proteins (CRABP-I and CRABP-II) which bind RA [277]. Moreover, these retinoid-binding proteins may control the metabolism of retinoids by delivering retinoids to various metabolic enzymes [282, 275]. In particular, CRBP-2 may promote esterification by lecithin retinol acyltransferase (LRAT) of retinol to the retinyl ester [115]. CRABP-I has been suggested to promote RA catabolism, whereas CRABP-II may assist with the transport of RA into the nucleus [78].

RA has been shown to be the active metabolite of retinoids in most biological processes [57]. Synthesis of RA occurs via a two-step oxidation of retinol to RA with retinal as the intermediate product [233].

Several enzymes have been implicated in the metabolization of retinol to retinal in the first step. Among these are enzymes that belong to the short-chain dehydrogenase/reductase (SDR; in particular retinol dehydrogenase, RoDH) and alcohol dehydrogenase (ADH) families [275, 83]. The physiological relevance in RA synthesis of ADH-1,-3, and -4 has been demonstrated by null-mutant mice studies. Adh3- and Adh4-disrupted mice, when subjected to vitamin A deprivation during gestation, suffer increased post-natal lethality [77]. Thus, ADH-3 as well as ADH-4 can be considered essential for RA production under vitamin A deficiency [264]. Additionally, ADH-3 was shown to be essential for growth under a normal diet [264]. Adh1-disrupted mice have reduced retinol to RA metabolism, without noticeable alterations [263].

The second step of RA synthesis is catalysed by aldehyde dehydrogenases (ALDHs) [83]. Importance of ALDHs in RA synthesis was demonstrated by disruption of Raldh2 in mice, which results in multiple brain abnormalities and embryonic lethality soon after RA synthesis should start [278, 246].

RA catabolism, also essential for a proper retinoid metabolism, involves RA inactivation by cytochrome P450s (CYP26s, in particular CYP26A1, CYP26B1, and CYP26C1) [5]. Null mutation of CYP26A1 results in abnormal CNS development and death soon after birth [5, 325].

The most important mechanism of RA activity involves gene expression regulation. RA exerts its effect on gene transcription by binding to specific nuclear receptors that are ligand-activated transcription factors [57, 128]. The RA signal is transduced by two nuclear receptor families: retinoid X receptors (RXRα, RXRβ, and RXRγ) are activated by the 9-*cis* RA isomer, and RA receptors (RARα, RARβ, and RARγ) by either the 9-*cis* RA or the all-*trans* RA isomer [57]. These receptors indeed mediate the functions of vitamin A during development, as has been demonstrated by mice lacking functional retinoid receptors which present multiple developmental defects, including those previously observed during vitamin A deficiency [406, 215, 242]. Interestingly, these mice also display behavioral abnormalities, such as spatial learning and memory deficits [61].

The retinoid nuclear receptors function as RAR-RXR heterodimers which, when active, bind to RA response elements (RARE) present in the regulatory

region of multiple genes, modulating their expression. The expression of more than 532 genes is influenced by RA availability, with RAREs being identified in around 30 genes [23]. Many of these genes are not the direct targets of retinoids but are rather modulated by other transcription factors, encoded by genes whose expression is regulated directly by retinoids [23]. Other types of indirect regulation have been proposed, including RA's ability to activate nuclear receptor dimers other than RAR-RXR. It has been noticed that RXRs are general partners for several nuclear receptors other than RARs, including peroxisome proliferator-activated receptors (PPAR), vitamin D receptor, orphan nuclear receptors (namely NR4A2), and thyroid hormone receptors (TR). RXR seems not to require RA binding to influence co-receptor action, as long as the appropriate ligand is bound to the co-receptor, but activated RXR may enhance action of a heterodimer pair [216] or even activate a partner without ligand [332].

1.2.3 Influence of thyroid hormones on brain function and behavior

Thyroid hormones (THs) affect diverse biological processes and are important for the functions of various organs through their influence on cellular metabolism [416]. Although TH affects the function of diverse organs in adults, the most important effects of TH are probably the ones exerted during growth and development [189]. This is manifest in the widespread clinical effects of insufficient supply of TH to the developing brain [268, 19, 189, 58, 72, 120].

Several lines of evidence have shown an association between early alterations of thyroid function and neurodevelopmental disorders [379, 298, 356, 116, 104]. The severity and irreversibility of CNS damage is dependent on the degree of TH abnormality, the time during development at which it occurs, and its duration. In particular, serious TH deficiency during pregnancy may result in spontaneous abortion, stillbirth, and congenital abnormalities [85]. Maternal TH deficiency may also lead to cretinism, a serious form of mental and physical retardation. More moderate TH deficiency leads to less visible, yet pervasive, mental impairment that reduces intellectual capacity [346, 189].

The importance of moment, duration, and magnitude of the TH abnormality is also borne out by effects of TH administration. Cretinism can result from maternal TH deficiency that affects the fetus before its own thyroid becomes functional. The damage is permanent and can not be reversed by post-natal TH supplementation. On the other hand, cretinism can also be caused by inadequate production of TH by the fetus itself, in which case it can be prevented if TH supplementation is initiated shortly after birth [73]. Mental impairment due to TH deficiency during gestation can be prevented by maternal administration of TH before mid-gestation. Organ function abnormalities in adults with hypo- and hyperthyroidism are reversible to a large degree by TH supplementation or inhibition of thyroid gland function, respectively.

Because of these intense effects of THs, and because TH exerts its effects through modulation of the expression of several genes, one can speculate that abnormal regulation of gene expression in the brain could be a key element in the explanation of many neurodevelopmental defects, among which schizophrenia [104].

1.2.4 Thyroid hormone metabolism

THs are the only hormones in vertebrates that contain iodine. Iodine is an essential element which can not be synthesised by the body and needs to be acquired through diet. Although the necessary amount of daily intake of iodine is small (ranging from 50-1000 micrograms per day), the scarcity of iodine in landlocked areas leads to insufficient dietary supply in many parts of the globe [385, 218, 390]. Without iodine the biosynthesis of THs is interrupted [53].

Figure 1.4 presents a schematic overview of the TH metabolism. A large percentage of the ingested iodine is absorbed through the small intestine and transported to the thyroid gland, where it is concentrated to meet TH requirements [53]. The first step in the TH synthesis is the activation of the *thyroglobulin* gene. Thyroglobulin synthesis is up-regulated by thyrotropin, or thyroid-stimulating hormone (TSH) [86]. After being produced, thyroglobulin undergoes a series of posttranslational processing steps, including iodine in-

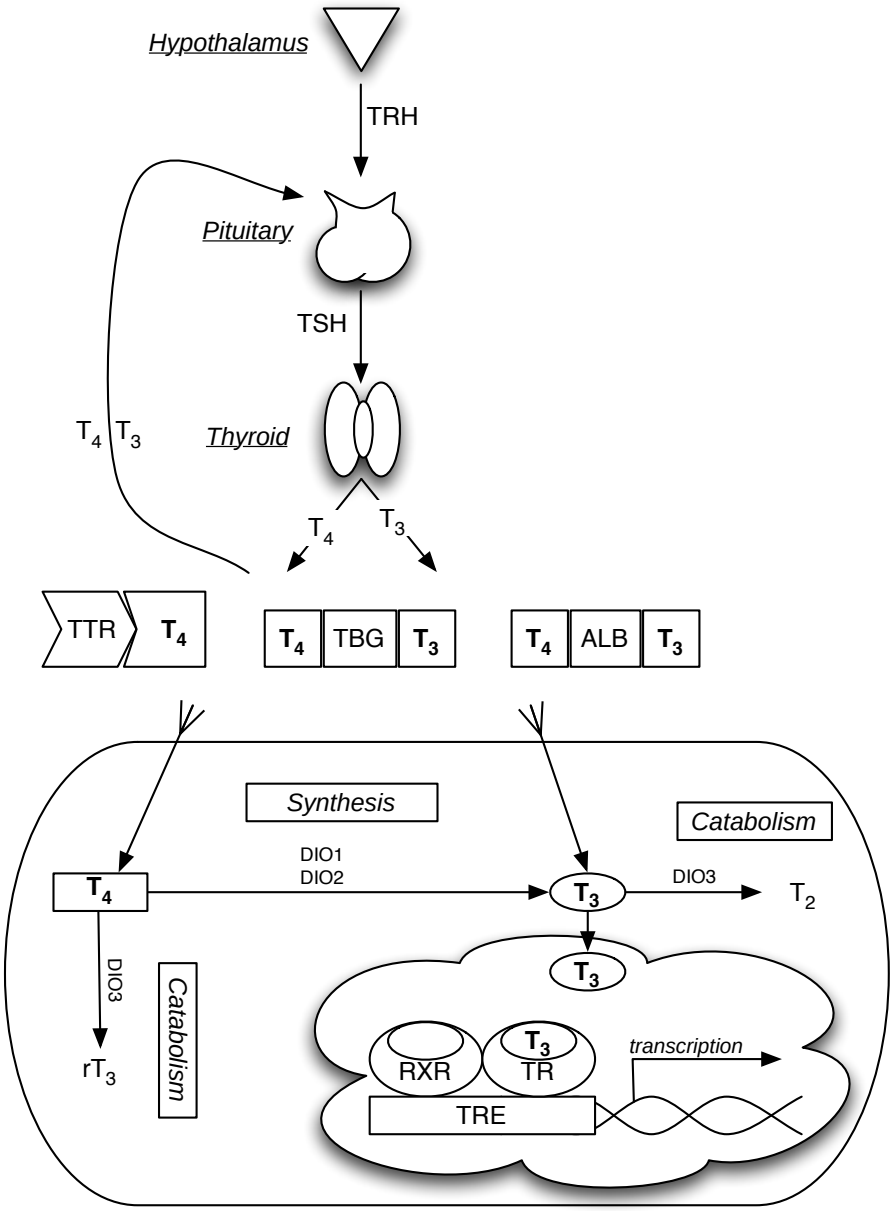

Figure 1.4: Schematic representation of the thyroid hormone metabolism. TH synthesis is regulated by the hypothalamus-pituitary-thyroid axis via a feedback mechanism involving T_3 and T_4. After secretion by the thyroid gland into circulation, THs are carried by plasma proteins, such as transthyretin (TTR), thyroxine-binding globulin (TBG), and albumin (ALB). After release from these carries, TH enters the cell, where the T_4 form is deiodinased into T_3 by DIO1 and DIO2. Subsequently, T_3 is either inactivated to T_2 by DIO3, or it crosses the nucleus membrane to bind to its nuclear receptor (TR). TR often forms heterodimers with retinoid X receptors (RXR). The hormone-activated receptor binds to TH response elements (TREs) to alter rates of gene transcription and consequently levels of mRNA.

corporation into the tyrosyl residues. Subsequently, a coupling reaction between pairs of iodinated tyrosine in the thyroglobulin molecules occurs to form thyroxine (T_4), and triiodothyronine (T_3) [86]. The posttranslationally modified thyroglobulin containing TH is stored. When needed, TSH stimulation of the thyroid gland results in thyroglobulin cleavage leading to the generation of free THs and the complete degradation of the protein [86]. T_4 is the main product of the thyroid gland. About 10% of T_4 is converted within the thyroid gland to T_3 through the action of deiodinases, a family of selenoproteins [367, 188]. Both T_3 and T_4 are then secreted into the circulation. It is estimated that in average a person with a normal thyroid function secretes 94-110 μg of T_4 and 10-22 μg of T_3 daily [102].

THs are insoluble and, once released, are rapidly bound to plasma proteins. In a normal person, less than 0.1% of the total serum T_4, and 0.3% of the serum T_3 are present in unbound form [62]. The major serum TH-binding proteins are thyroxine-binding globulin (TBG) that carries approximately 70% of TH, transthyretin (TTR) which binds about 10% to 15% of the hormone, and albumin that binds 15% to 20% of T_4 and T_3. Several other serum proteins bind T_4 and T_3 as well as reverse T_3 (rT_3), but their contribution to the overall hormone transport seems negligible in both physiological and pathological situations. In particular, lipoproteins transport a minor fraction of T_4 and T_3 through specific interaction with various apolipoproteins (for review see [314]). In the CSF, TTR is the major TH-binding protein.

T_4, the most common form of TH in circulation, is carried to different organs and tissues where it is locally converted into T_3. A large proportion of the T_3 in circulation is produced outside of the thyroid gland in the liver and kidney by the action of type I deiodinase (DIO1). In the brain, T_3 is produced locally by the action of type II deiodinase (DIO2). Inactivation of TH is carried out by the action of type III deiodinase (DIO3), that converts T_4 into rT_3 and T_3 into T_2 that have little or no biological activity, and by glucoronosyltransferase and sulfotransferase enzymes that participate in TH elimination process [207].

The effects of THs are mostly mediated by nuclear TH receptors (TRs)

which are T_3-responsive transcription factors. The active TRs bind to their specific TH response element (TRE) in the regulatory regions of target genes in two different ways, as homodimers or as heterodimers with RXR, activating or repressing the expression of several target genes [416, 104]. THs have been shown to directly control the expression of more than 50 genes [100].

No tissue or organ other than the thyroid has been shown to produce TH. Its synthesis is regulated by the hypothalamus-pituitary-thyroid axis, via a feedback mechanism depicted in the upper half of Figure 1.4.

The hypothalamus produces thyrotrophin-releasing hormone (TRH) which is then transported to the pituitary gland, promoting production and release of TSH. Subsequently, TSH stimulates the thyroid gland to produce TH. The increase of TH levels in circulation has a negative feedback effect on the pituitary, reducing its response to TRH. Conversely, when circulation levels drop, the pituitary's response to TRH increases, leading to increased TSH release.

Additionally, this regulatory loop is affected by internal and external factors that alter the rate at which TSH is secreted [329]. For example, exposure to low environmental temperatures may stimulate the release of TSH by adjusting TRH secretion. Increased levels of somatostatin, dopamine, cytokines, and glucocorticoids reduce secretion of TSH. Estrogens have the opposite effect, increasing TSH.

As we discussed earlier, inadequate supply of iodine leads to inadequate TH production and, therefore, TH function ultimately depends on an adequate supply of iodine to the gland. Inadequate supply of iodine raises the levels of TSH secretion which in turn stimulates TH synthesis and release. In particular, intrathyroidal deiodination increases when the thyroid is stimulated by TSH, leading to the release of a large amount of T_3, which is the biologically active metabolite of THs [53].

Levels of free TH in serum are directly related to the amount of hormone entering the cell. Therefore, concentration of the free T_4 and T_3, rather than the total TH, is usually a more accurate indicator of the activity level of TH-dependent cell processes [314].

1.2.5 Retinoids and thyroid hormones as biochemical basis for the neurodevelopmental hypothesis of schizophrenia

Many similar characteristics exist between the modes of action of retinoids and THs, and between their metabolisms. The main mechanism of action of both is their regulatory influence on the expression of several target genes upon interaction with nuclear receptors. As we discussed before, TTR is a major carrier of TH in the CNS and also forms a complex with retinol-binding protein. Therefore TTR participates in the homeostasis of both retinoids and THs. Interestingly, besides belonging to the same family of nuclear receptors, RXR and TR often heterodimerize. Both retinoids and THs are involved in the regulation of processes such as differentiation of the cerebellum, axonal migration and myelination [317], and the control of lateralization and symmetry of the embryo [383, 382]; processes that have been implicated in schizophrenia [293]. Among the genes regulated by retinoids and THs are those that encode myelin basic protein [317], dopamine receptor D2 [327] and neuregulin 1 receptor, ERBB4 [285], which have all been implicated in schizophrenia.

In view of these similarities, the previously proposed retinoid hypothesis of schizophrenia [125] has recently been extended to THs [293].

Since retinoids and THs are essential for numerous neurodevelopmental processes associated with schizophrenia, abnormal function of these two “endocrine systems” could play a role in the disease etiology. The enzymes and other proteins that regulate both TH and retinoid metabolism are genetically determined, but their availability is dependent on supply of vitamin A and iodine acquired from the environment. Therefore, retinoids and THs might offer a candidate functional link between the identified genetic and environmental risk factors in schizophrenia (see Figure 1.5).

The search for evidence that would support the involvement of TH and retinoids in schizophrenia could include studies of plasma TH and retinoid levels in patients compared with controls, but also genetic association studies. The effects of retinoids and THs are dependent on the quantity of retinoids and TH reaching the tissues and the availability of the correspondent recep-

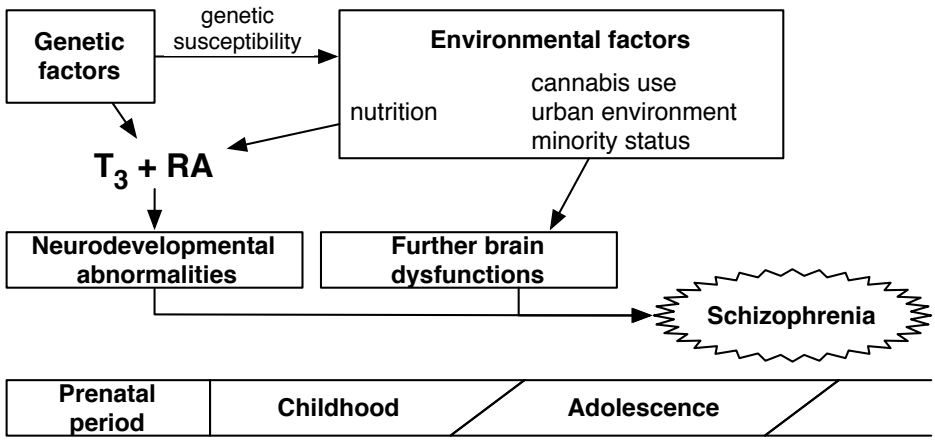

Figure 1.5: Retinoids and thyroid hormones may provide a functional link between genetic and environmental risk factors in the neurodevelopmental hypothesis of schizophrenia.

tors in the cell nuclei. Retinoid and TH metabolism could be altered by the presence of genetic variants in genes which would result in abnormal activity of a transporter, an enzyme, or other proteins which regulate their metabolism.

DNA variants that occur frequently in the population are known as "polymorphisms" and they are linked with modest but true biological effects. Their abundance in the human genome as well as their high frequencies in the human population justify the idea that they can explain variations in the risk to common diseases, as we will explain in Section 1.3.2.

1.3 Molecular genetics studies in schizophrenia research

Identification of schizophrenia susceptibility genes has proven to be an extremely difficult task. This is due to clinical heterogeneity, a complex and uncertain mode of inheritance, effects of incomplete penetrance, the involvement of non-genetic factors, and the likely large number of susceptibility genes contributing each with a small risk only. Currently, linkage and association studies together with robust statistical methods, as well as gene expression analysis are being used to identify schizophrenia genes.

1.3.1 Linkage studies

The literature on schizophrenia linkage studies is particularly extensive and complex, replete with attractive original observations and subsequent studies that fail to replicate initial findings. These apparent discrepancies may, in fact, reflect the complex nature of schizophrenia.

Classical linkage studies analyze the segregation of markers along with the disease phenotype and require large pedigrees with several affected members. If, in a family, all affected individuals present a particular marker pattern, different than the ones showed by unaffected individuals, the chromosomal region in the vicinity of the marker represents a potential region for mapping disease-related genes. Subsequently, more markers must be tested to narrow the region of interest and to eventually identify the causative gene. Application of molecular biology techniques in combination with statistical linkage analysis has been highly effective in identifying rare variants in high risk genes, such as the ones involved in single-gene, Mendelian disorders [46].

Because of their power in identifying *loci* for classical Mendelian disorders, linkage studies have been extensively used in the attempt of identifying genes underlying multifactorial (non-Mendelian) diseases [46]. In these complex traits, although it is unlikely that uncommon high risk genes are responsible for disease susceptibility in the general population, they can possibly be identified in specific samples of large families with multiple affected members. Several strategies have been used in schizophrenia linkage studies in order to improve the discovery of positive results. Nuclear families and sib-pair linkage studies have been performed, both in general populations and in geographically or ethnically isolated populations, in which there may be less genetic heterogeneity due to a founder effect. To avoid the specification of the mode of inheritance, nonparametric statistical analyses have been employed instead of parametric ones [371]. To avoid clinical heterogeneity, the genetic study of schizophrenia can be simplified by focusing on specific inherited features of the disorder, such as the endophenotypes mentioned earlier (see Section 1.1.2). Despite all the efforts, and in contrast with other complex disorders, such as Alzheimer's disease or breast cancer, linkage studies so

far have failed to pinpoint large single-gene effects. This is actually consistent with the epidemiological evidence, suggesting that the existence of a single locus accounting for most of the genetic susceptibility in schizophrenia is very unlikely. In addition, the frequent non-replication of initial reports has been interpreted as an indication that linkage analyses are not reliable in the detection of genes of small effect [313].

Technological improvements in the identification and genotyping of polymorphic DNA markers have increased the ability to genetically map complex disorders. Large collaborative efforts have been undertaken in order to study much bigger samples, which facilitates the detection of susceptibility genes of moderate effect [36]. While the number of genome scans increases as well as the sample sizes used, replicated linkage to several chromosomal regions are starting to accumulate. Recently, two meta-analyses of all published whole-genome linkage scans, using different statistical methods, have obtained somewhat overlapping results [22, 209]. The Badner and Gershon study supports the existence of susceptibility genes on chromosomes 8p, 13q, and 22q [22]. The Lewis meta-analysis also identifies 8p, and 22q as susceptibility regions as well as 1q, 2q, 3p, 6p, 11q, 14p, and 20q [209]. Interestingly, in this last study the stronger linkage was found in chromosome 2q, a region that had not previously been reported.

The 22q11 region has received particular interest based on estimations that around 25% to 30% of velo-cardio-facial syndrome (VCFS) patients, which present a deletion on this chromosome region, develop schizophrenia as they reach adulthood [25, 302, 274]. The high prevalence of schizophrenia in this group and the observation that 22q11 deletion is present in 2% of schizophrenic patients [178] along with reports of linkage to 22q11 [22, 209] suggest that this chromosome region might harbor a gene or genes relevant to the etiology of schizophrenia [16].

In summary, the linkage data in schizophrenia demonstrate several features that were already predicted on the basis of genetic epidemiological findings [310]. Existence of a major risk locus is highly unlikely, and the level of statistical significance implicating a number of regions is consistent with the presence of several genes of moderate effect. Finally, the chromosomal re-

gions of interest are typically broad.

Positive linkage results are only proved to be true when the disease gene(s) are identified. To identify genes in regions for which positive linkage results have been obtained, association studies are increasingly performed to follow up on linkage evidence.

1.3.2 Association studies

The use of association rather than linkage studies seems a simpler and more powerful approach to detect genes of small effect [261, 312]. The common approach to detect association is the case-control design, in which the frequency of a specific genetic marker is compared between affected and unaffected individuals. The main advantage of this design is that it makes use of unrelated individuals, which are relatively easy to recruit. The main weakness is the high rate of false-positives that can be obtained due to inappropriate selection of controls [153]. This phenomenon may occur in the case of population stratification (or admixture), where cases and controls, taken from a population in which different genetic backgrounds are present, do not have the same proportion of individuals from the various backgrounds. In this situation, false positive results will be obtained if the trait is more prevalent in one of the genetic groups and the frequency of genetic markers is not the same in all groups.

This limitation of case-control association studies prompted the development of family-based designs that use unaffected siblings as controls or reconstruct the "control" using the non-transmitted parental alleles. However, familiar relations imply that cases and "controls" will present high genetic similarity (overmatching) and, consequentially, family-based studies are less powerful when compared to well-designed case-control studies.

Association studies have been frequently applied to the study of candidate vulnerability genes. These candidate genes can be selected for various reasons: because they are located on a chromosomal region previously implicated in the etiology of schizophrenia by linkage studies; their expression is altered in postmortem brain tissue of schizophrenic patients; they are implicated by animal models of schizophrenia; or, finally, they belong to metabolic

or signaling pathways hypothesized to be changed in schizophrenia.

Although no susceptibility gene for schizophrenia has consistently been detected, several strong candidate genes have recently been identified. Most of these genes are located on chromosomal regions previously implicated by linkage studies, such as *neuregulin 1* (*NRG1*) in chromosomal region 8p21-22 [351], *dysbindin* (*DTNBP1*) in 6p22.3 [353], *D-amino acid oxidase activator* (*DAOA* or *G72*) in 13q22-34 [64], *RGS4* in 1q21-22 [63], *calcineurin gamma subunit* (*PPP3CC*) in 8p21.3 [114], *trace amine receptor 4* (*TRAR4*) in 6q23.2 [82], and *catechol-O-methyltransferase* (*COMT*) and *proline dehydrogenase* (*PRODH*) in 22q11 [212, 342]. Many of these studies have been replicated. In addition, some of these genes were found to be differentially expressed in postmortem schizophrenic brains as is the case for *NRG1* [199], *DTNBP1* [360, 395], *RGS4* [253], and *COMT* [230]. To make a strong case, functional studies must be carried out for each implicated gene, in order to explore whether it affects developmental and behavioral characteristics of relevance to schizophrenia. For example, for *PRODH* and *calcineurin*, transgenic mice have displayed changes in “schizophrenia-like” behaviors [417, 255, 122].

It should be noted that none of these promising candidate genes, with the exception of *COMT*, have been linked with a DNA variant capable of changing gene function or expression. Even for the two genes with highest replication ratios (*DTNBP1* and *NRG1*), important questions remain. No single high-risk haplotype is associated across all samples. This can possibly be caused by the presence of multiple susceptibility alleles or by the fact that a single risk allele is carried on a large diversity of haplotypes, even in closely related populations. It is possible that multiple variants in a particular gene all cause similar liability to the disease. If, in fact, schizophrenia has appeared early in the evolution of modern *Homo sapiens* [30], a single primordial variant may have been extensively shuffled by recombination, yielding haplotypes that are differentially represented in different populations. Evidence exists supporting such haplotype differences for some genes even among closely related Northern European groups [306].

An exciting observation is that most of the associated genes encode pro-

teins that can be directly related to the schizophrenia pathophysiology via modulation of glutamatergic neurotransmission [142].

1.3.3 Gene expression studies

The recent development of commercial RNA expression platforms (microarrays) allows the screening of tens of thousands of genes in a relatively short period of time, requiring relatively small amounts of biological material. These microarray studies provide a high-throughput methodology to identify differentially expressed genes between ill and healthy persons, and to measure the activity and the effect of several drugs or pathogens on different types of cells and tissues. Changes in mRNA expression can result in phenotypic differences, though this is not always the case. Altered gene expression does not always correspond to abnormal protein levels, even when measured in the same group of brains [380, 187].

Microarray studies are primarily a screening tool. Their results must be validated by more laborious methods such as *in situ* hybridization and real-time polymerase chain reaction, which provide higher resolution. At the level of individual genes little agreement has been achieved between microarray studies. There are even cases of genes whose expression have been reported increased in some studies and decreased in others [250, 134]. These discrepancies can in part be explained by technical differences between studies, such as the selection of genes present in the array, quality of brain tissue, number of individuals studied, microarray producer, and bioinformatics protocols applied in the analysis.

Several studies analyzing genome-wide expression in the brain have identified a high number of genes differentially expressed in schizophrenic patients compared with mentally healthy individuals [380, 252, 247, 134]. Biological validation of microarray results must be conducted to investigate whether the alteration in gene expression actually represents the cause of the disease, or results from the disease pathophysiology or from chronic medication.

Apart from identifying individuals genes, microarray studies have the capacity to detect alteration in expression patterns of multiple genes, leading

to the identification of relevant metabolic and signaling pathways involved in schizophrenia. In fact, a number of gene networks have been detected to be affected in schizophrenia. Among these are oligodendrocyte- and myelin-related genes [364], and networks that control pre-synaptic function [252], neurochemical impairment of synapses [166], synapse formation and pruning [251], neuronal migration [65], gene transcription regulation [365], and G-protein signaling [251]. These altered pathways, although requiring biological and clinical validation, provide insights into the schizophrenia etiology, and give rise to new working hypotheses.

In summary, the combination of linkage, association, expression, and functional studies is expected to provide answers in the search for the genes underlying the schizophrenia etiology.

2

Research objective and strategy

The general objective of the work reported in this thesis was to investigate whether retinoids and THs are related to the pathophysiology of schizophrenia. In other words, to search for evidence that may help to support or disprove the theory explained in Section 1.2. Our search strategy consisted of the following steps.

Propose functional candidate genes. To propose candidate genes, we studied literature on genes related to TH and retinoid metabolisms, including their transporters, their nuclear receptors, enzymes involved in their synthesis, and on genes whose transcription they regulate. In particular, we focused on genes that have somehow been functionally related to schizophrenia.

Search for DNA variants in selected candidate genes. To find DNA variants in the selected genes we searched for known variants reported in the literature or deposited in human genome databases. Additionally, in order to find novel variants, we applied molecular techniques on DNA samples of schizophrenic patients, such as Single Strand Conformational Polymorphism (SSCP) analysis and sequencing.

Genotype selected variants in appropriate samples. Based on characteristics of the DNA variants found, such as their frequency and their pair-wise linkage disequilibrium (LD), we selected several variants in each candidate gene. We then genotyped the selected variants in samples from different geographic locations and corresponding to different study designs (case-control and parent-patient triples).

Analyze the obtained data to establish whether association exists between the variants and schizophrenia. We applied appropriate statistical tests to the obtained genotypic and corresponding diagnostic data to establish whether significant associations exist between particular DNA variants and schizophrenia.

In the remainder of this thesis, we report on the study of each of the selected

Table 2.1: Studied candidate genes.

Gene	Transport	Receptor partner	Transcription regulated by	Schizophrenia abnormality
NR4A2	-	RXR	-	Dopamine transmission
PTGDS	TH Retinoid	-	TH Retinoid	Lipid metabolism
TTR	TH Retinoid	-	-	(Behavior)
NRGN	-	-	TH Retinoid	Glutamate transmission

functional candidate genes (see Table 2.1). For each of these genes, we studied the association of several DNA variants with schizophrenia.

The *NR4A2* gene, studied in Chapter 3, forms heterodimers with retinoic X receptor (RXR) and has been related with dopamine transmission, known to be abnormal in schizophrenia. For this gene, six rare variants have been described in the literature, and we investigated their presence in a Portuguese and a Brazilian sample of schizophrenic patients and controls.

The lipocalin-type of prostaglandin D2 synthase (PTGDS) is not only transporter of both TH and retinoids, but its expression is also regulated by them. PTGDS is related to lipid metabolism, which is known to be abnormal in schizophrenia, and is studied in Chapter 4. We studied the possible association of three variants of *PTGDS* with schizophrenia in two case control samples, one from the Portuguese mainland and one from Brazil, and in a parents-patient sample from the Azorean islands.

TTR is a transporter of both THs and retinoids, and is studied in Chapter 5. We searched for association of two polymorphisms in the *TTR* gene with schizophrenia in the same three samples. We also analyzed the circulating levels of TTR itself and of RBP, which is transported by TTR.

The *Neurogranin* gene (*NRGN*) is studied in Chapter 6. The expression of *NRGN* is regulated by THs and retinoids, and it is related to the transmission of

glutamate, believed to be implicated in schizophrenia. We studied association of three variants of *NRGN* to the disease in the two case-control samples and in the parents-patient sample.

As we will see, the study of *NRGN*, in particular, has provided interesting results in the context of the schizophrenia etiology.

Apart from association studies, evidence for the involvement of retinoids and THs in schizophrenia could be obtained through studies of their plasma levels in patients and healthy individuals. Though not central to this thesis, we report on a preliminary study of this kind in Chapter 7, where serum levels of five TH indicators (total and free T_4, total and free T_3, and TSH) are measured in the male-subsample from the Portuguese mainland.

In Chapter 8, we summarize and discuss the relevance of the various results obtained, and we provide suggestions for future work.

3

Nur-related receptor 1 and schizophrenia

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Abstract

The present study investigates the association of mutations in the nuclear receptor *NR4A2* with schizophrenia. The human Nur-related receptor 1, NR4A2, is an orphan nuclear receptor that can be constitutively active as a transcription factor and for which no natural ligand has yet been identified. Alone or with the retinoid X receptor, RXR, NR4A2 influences the expression of several genes important for human brain development and regulation. In the absence of Nurr1 (the mouse homologue to human NR4A2), ventral mesencephalic dopaminergic mouse neurons evidence severe developmental failure, a condition that is lethal soon after birth. Nurr1 involvement in the dopaminergic system makes it a good candidate for study in neuropsychiatric disorders such as schizophrenia and Parkinson's disease. Evidence by others support this hypothesis: 1) mapping of the *NR4A2* gene to chromosome 2q22-23, a region with suggestive linkage to schizophrenia and 2) identification of mutations in patients with schizophrenia (c.366-369delTAC, c.308A>G, c.-469delG), manic depression (c.289A>G) and familial Parkinson's disease (c.-291delT, c.-245T>G). To further extend these observations, we searched for all these mutations in 176 Caucasian Portuguese and 82 Caucasian Brazilian subjects with lifetime diagnosis of schizophrenia. The study failed to identify any of the described mutations in patients or controls. Nevertheless, these negative results do not exclude altered expression of nuclear receptors in schizophrenia or the presence of other mutations.

3.1 Introduction

Schizophrenia is a chronic psychotic disorder of unknown etiology. Twin and family studies demonstrate a genetic basis for the disease [372]. Several genome wide studies identified chromosome *loci* consistently linked to increased susceptibility to schizophrenia in different populations [209]. Despite the evidence for genetic predisposition, the lack of concordance in monozy-

gotic twins implies that an epigenetic environmental effect is required for disease onset [372]. For this reason, interest has been raised in hypotheses bridging both genetic and environment-related mechanisms. Environmental factors such as hormones and vitamins interact with different nuclear receptors and interfere with the transcription of several genes in a developmental regulated fashion. Among these are the retinoids, considered good candidates because of genetic linkage studies implicating schizophrenia with dysregulation of the retinoid cascade and/or genes whose expression they regulate [125]. The genes for dopamine-2 receptor, synapsin and dopamine -hydroxylase are among those whose expression requires activation by retinoids [327, 184, 185, 23]. Pharmacological, histological and brain imaging data have for long implicated dopaminergic dysfunction in the etiology of schizophrenia. Retinoid availability can, therefore, interfere with susceptibility to schizophrenia, either directly, through the dopamine system, or indirectly through molecules involved in its development and/or regulation. Interestingly, retinoid analogs have been suggested in the treatment of schizophrenia, alone or in combination with dopamine receptor agonists [66].

RA receptors belong to the steroid/thyroid hormone nuclear receptor superfamily that includes several orphan receptors for which no ligands have been identified. These receptors often act as heterodimers greatly increasing the complexity of gene regulation. NR4A2, one of these orphan nuclear receptors, is known to regulate transcription of target genes in two different ways: alone, as a monomer, or as a partner with the retinoid X receptor (RXR) [295, 391].

Nurr1 (the mouse homologue to human *NR4A2* gene) mRNA is expressed very early in the ventral midbrain [419, 418] and disruption of its expression is responsible for massive failure to generate dopaminergic neurons in the midbrain and causes death soon after birth [418, 328]. The lack of dopaminergic neurons in the midbrain seems to be related with the inability to express tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis [419]. Furthermore, Nurr 1 seems to retain its role in mature dopaminergic neurons, since its expression continues over adulthood and is extremely impaired after ventral mesencephalic dopaminergic neurons injury [419].

These observations increased the interest in identifying precise molecular and biochemical mechanisms of behavior regulation by NR4A2 in psychiatric and neurological disorders. Furthermore, the 2q22-q23 chromosome region that harbors the *NR4A2* gene has suggestive linkage with schizophrenia in various populations [260, 408, 75, 209]. In accordance to this hypothesis several groups have looked for mutations in the *NR4A2* human gene that could increase susceptibility to schizophrenia. Up to date, two deletions and one missense mutation have been described in schizophrenic patients and one missense mutation has been found in manic-depressed individuals [44, 60] while, recently, two mutations in the untranslated region have been identified in patients with familial Parkinson's disease [205]. These findings prompted us to investigate all these *NR4A2* mutations in a large sample of Portuguese and Brazilian schizophrenic patients. Patients fulfilled DSM IV lifetime diagnosis of schizophrenia, confirmed by OPCRIT diagnostic algorithm. None of the mutations described in the literature were identified in this group of patients.

3.2 Materials and Methods

The sample consisted in 176 Portuguese (age range 15-74, average 34.0; 67 female and 109 male) and 82 Brazilian (age range 18-63, average 34.3; 10 female and 72 male) schizophrenic patients and 105 Portuguese (age range 19-79, average 35.5; 53 female and 52 male) and 85 Brazilian (age range 19-58, average 32.9; all men) mentally healthy individuals. All subjects were unrelated Caucasians. Patients gave informed consent for the study, and ethic committees of both institutions approved the study. All patients (Portuguese and Brazilian) were classified by the OPCRIT system (Operational Checklist for Psychotic Disorders) [237]. OPCRIT automated system gathered information from all case records, including medical, nursing, social work and occupational therapy notes together with data from clinical interview with patient and relatives. Additionally, Portuguese schizophrenic patients received lifetime diagnosis using DIGS (Diagnostic Interview for Genetic Studies) as previously described [283]. Controls were from European origin or descent and

free of any lifetime diagnosis of major mental illness and physical illness. Portuguese controls received DIGS assessment and Brazilian controls were selected among blood bank volunteer donors documented to be free of chemical dependence [308]. All interviews and diagnostic formulations were performed by one of the authors. Venous blood was drawn from all subjects and DNA extracted using standard salting-out procedures.

For genotyping the c.-245T>G, c.289A>G, c.308A>G mutations and the deletion c.-469delG, we followed PCR conditions previously described [44, 60, 205]. PCR products were analyzed on gel electrophoresis after digestion with the restriction enzymes Ava II (Fermentas, Vilnius, Lithuania), ApaL I (Fermentas), Tse I (New England BioLabs, MA, USA) and Cfr I (Fermentas), respectively. The c.366-369delTAC deletion was screened by Single Strand Conformation Polymorphism (SSCP) on the PCR product amplified with the primers (5´-3´) CTTGTACCAAATGCCCCTGT and GAGACTGGCGTTTTCCTCT. Electrophoresis on 10% non-denaturing polyacrylamide gel with 2.5% glycerol was performed at 500 V for 2.5-3 hours. Temperature was strictly maintained at 14ºC. Samples from patient carriers of the mutations c.366-369delTAC, c.289A>G and c.308A>G were used as controls.

Search for the c.-291delT on the PCR product amplified with primers previously described [205] was done by SSCP analysis on MDE gel (BMA, Rockland, USA) run at 4, 10 or 25ºC.

3.3 Results

Using SSCP analysis, we searched the deletions c.366-369delTAC and c.-291delT, looking also for other possible mutations [384] on the same PCR product. Figure 3.1 shows the SSCP migration pattern of a c.366-369delTAC deletion carrier. None of the samples tested revealed the presence of mutated alleles.

For the c.-291delT deletion we analyzed one subpopulation of 60 Portuguese schizophrenic patients and ran the SSCP at 3 different temperatures to increase the rate of detection. Again, we failed to identify any mutation in

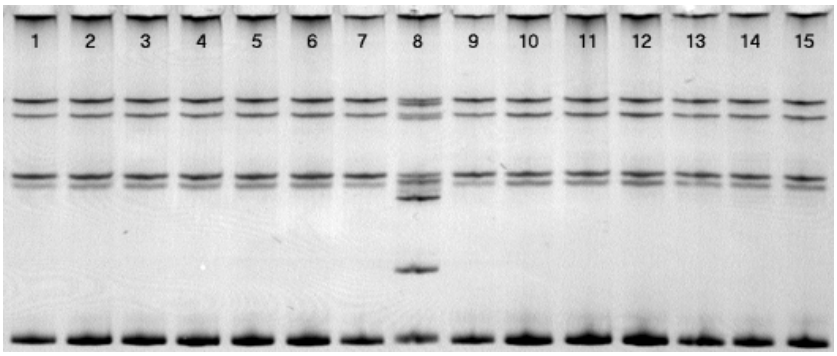

Figure 3.1: SSCP of the *NR4A2* exon 3 PCR fragment. On lane 8 run a sample containing the c.366-369delTAC mutation. Lanes 1-6 correspond to Brazilian schizophrenic patients and lanes 9-15 to Brazilian mentally healthy individuals.

the PCR product containing the position c.-291. Analysis of the mutations c.-469delG, c.-245T>G, c.289A>G and c.308A>G was done by digestion with the enzymes Cfr I, Ava II, ApaL I and Tse I for which a new restriction site is present in the mutated allele. None of the samples, from schizophrenic or mentally healthy individuals, contained any of the mutations screened.

Table 3.1 is a summary of all studies, including ours, in which *NR4A2* mutations described in diseases have been investigated.

3.4 Discussion

In the present study we investigated, for the first time, the presence of two *NR4A2* mutations recently identified in familial Parkinson's disease [205] in 258 schizophrenic patients and 190 mentally healthy individuals. We also searched for mutations in *NR4A2* previously described in patients with schizophrenia and manic-depression [44, 60]. No mutation was found either in patients or controls. Given the complex etiology of schizophrenia and the failure to identify a single causative gene, it is unlikely that any individual mutation will be strongly represented in the patient's population [56]. In the case of transcription factors such as nuclear receptors, several different mutations can impair proper function and influence the appropriate expression of several genes. Therefore, for any new mutation found it is important to increase

Table 3.1: *NR4A2* Mutations Detected in Patients With Schizophrenia, Manic Depression, and Parkinson's Disease. Represented is the number of individuals in which *NR4A2* mutations were found among all patients tested. Transcription activity was measured by *in vitro* studies (↓=Decreased, ↑=Increased, ? =Not determined).

Mutation	Location		Population
c.-469delG	Promoter	?	2/176 Han Chinese schizophrenic [60] 0/176 Caucasian Portuguese schizophrenic 0/82 Caucasian Brasillan schizophrenic
c.-291delT	Exon 1, untranslated	↓	8/107 familial & 0/94 sporadic Parkinson's (mostly Caucasian) [205] 0/60 Caucasian Portuguese schizophrenic
c.-245T>G	Exon 1, untranslated	↓	2/107 familial& 0/94 sporadic Parkinson's (mostly Caucasian) [205] 0/176 Caucasian Portuguese schizophrenic 0/82 Caucasian Brasilian schizophrenic
c.289A>G	Exon 3, coding	↓	0/135 Swedish schizophrenics [44] 0/160 North American Caucasian schizophrenics [44] 0/70 Caucasian Swedish idiopathic Parkinson [44] 1/30 Caucasian Swedish manic depressed [44] 0/176 Caucasian Portuguese schizophrenic 0/82 Caucasian Brasilian schizophrenic
c.308A>G	Exon 3, coding	↓	1/135 Swedish schizophrenics [44] 0/160 Caucasian North American schizophrenics [44] 0/70 Caucasian Swedish Idiopathic Parkinson [44] 0/30 Caucasian Swedish manic depressed [44] 0/176 Caucasian Portuguese schizophrenic 0/82 Caucasian Brasilian schizophrenic
c.366-369delTAC	Exon 3, coding	↓	1 childhood-onset/135 Caucasian Swedish schizophrenics [44] 0/160 Caucasian North American schizophrenics [44] 0/70 Caucasian Swedish Idiopathic Parkinson [44] 0/30 Caucasian Swedish manic depressed [44] 0/176 Caucasian Portuguese schizophrenic 0/82 Caucasian Brasilian schizophrenic

the size of the patient sample analyzed. The fact that we failed to identify the mutations previously described in *NR4A2* confirms that they are very rare.

Other studies have reported lack of association of *NR4A2* polymorphic variants in the promoter [52], 5´ and 3´ untranslated regions and intron 6 with schizophrenia [168, 170]. The human *NR4A2* gene exists as a single locus in human genome, covering 8.3 Kb in length, consisting of eight exons and is mapped to chromosome 2q22-23 [164, 366]. This chromosome region has been implicated in schizophrenia [209]. The mutations c.289A>G, c.308A>G and c.366-369delTAC, originate amino acid changes at the protein level (M97V, H103R, and Y122, respectively) and result in decreased in vitro transcriptional activity of NR4A2 dimers [44]. Decreased transcription activity

of the *NR4A2* is also described for the mutations in the 5´ untranslated region found in patients with Parkinson's disease [205]. Therefore, impaired transcription activation of downstream target genes such as tyrosine hydroxylase is expected in carriers of these mutant variants of *NR4A2*.

Studies in mice have revealed important functions for NR4A2 that clearly suggest its possible involvement in several disorders of the central nervous system in which the dopaminergic system has been implicated. Observations in Nurr1-null mice revealed that Nurr1 is requested for the formation of midbrain dopaminergic neurons [418, 328] and that tyrosine hydroxylase, the rate-limiting enzyme in the cathecolaminergic pathway, is absent in dopaminergic neurons [418]. On the other hand, mice lacking the D2 receptors for dopamine show increased Nurr1 expression in mesencephalic dopaminergic neurons, suggesting that actions mediated by D2 receptors might be a consequence of altered expression of Nurr1 [370]. In addition, Nurr1 enhances the transcription of the human *dopamine transporter* gene [323], one of the most specific phenotypic markers for dopaminergic neurons, and of the *tyrosine hydroxylase* gene [326, 184, 185]. Studies in Nurr1-null heterozygous mice show that Nurr1 increases spontaneous locomotor activity in response to stress [88]. The effect of amphetamines in Nurr1-null heterozygous locomotion remains controversial [88, 27].

These observations suggest that NR4A2 by itself, or through heterodimerization partners, may participate in diseases with altered dopaminergic function such as Parkinson's, schizophrenia and drug abuse. NR4A2 may also be implicated in some personality traits with increased vulnerability to stress [88] such as those in the schizophrenia phenotype spectrum. Both mutations associated with decreased or increased NR4A2 activity and with altered regulation of the *NR4A2* gene throughout development might be associated with disorders such as schizophrenia and Parkinson's. Future studies must address whether the expression of *NR4A2* is altered in the brain of schizophrenic patients or influences their response to alcohol exposure or drug treatment. Better understanding of the pathways involving NR4A2 might make it a potential target for therapy with drugs like 6-mercaptopurine analogs or even stem-cell transplants as recently suggested [286].

4

Lipocalin-type prostaglandin D2 synthase and schizophrenia

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Abstract

Several observations point to the involvement of disturbed lipid biology in schizophrenia. Reduced response to niacin flushing test, which involves vasodilatation induced by prostaglandin D2 (PGD2), is among the evidences, together with decreased CSF levels of lipocalin-type prostaglandin D2 synthase (PTGDS), the enzyme responsible for the synthesis of PGD2 in the brain. Since PTGDS is also a carrier for lipophilic molecules such as retinoids and thyroid hormones, altered PTGDS levels might influence both PGD2-mediated signaling, and vitamin A and thyroid hormone availability. To test whether genetic variants of PTGDS are involved in the etiology of schizophrenia, we searched for variants in the coding and regulatory regions of the gene. We identified four previously described polymorphisms. Using two case-control samples from Portugal and Brazil, none of the polymorphisms tested was associated with the disease. In addition, no transmission distortion was observed in an independent parents-offspring sample from the Azorean Islands. Our data do not support the involvement of the *PTGDS* gene in the etiology of schizophrenia.

4.1 Introduction

Schizophrenia is a common mental disorder affecting about 1% of the world's general population [171]. Family, twin, and adoption studies indicate the presence of a large genetic component [239]. However, genetic linkage studies have failed to identify a major risk chromosomal region [301]. The difficulty of finding *loci* or genes underlying schizophrenia suggests involvement of several genes, each conferring only a small degree of risk [347]. Association studies are generally accepted to be the most powerful method for detecting genes of small effect in complex disorders like schizophrenia [312].

Cumulative results support that schizophrenic patients have altered lipid metabolism [101], including increased phospholipids breakdown and decreased levels of polyunsaturated fatty acids, particularly arachidonic acid [414]. Arachi-

donic acid and its products (e.g. prostaglandins) are critical to various signaling pathways [35]. Interestingly, schizophrenic patients are reported to present reduced response to niacin [159, 393, 345]. Flushing in response to niacin (an acute allergic response) involves epidermal release of prostaglandin D2 (PGD2) [271, 270] which suggests the existence of an abnormal prostaglandin signaling. In addition, the flush response to niacin does not differ between medicated and unmedicated schizophrenics [338] and is also impaired in non-psychotic first-degree relatives of schizophrenic patients [386], suggesting that the alteration in niacin sensibility is a genetic trait independent of medication status. Underlying the abnormal niacin response might be decreased levels of arachidonic acid [101] or impaired activity of the enzyme responsible for the synthesis of PGD2.

There are two distinct types of enzymes, encoded by different chromosomes, responsible for PGD2 synthesis [376, 375, 374]. The hematopoietic-type PGD2 synthase, encoded on 4q22.3, is responsible for the production of PGD2 in the periphery and the lipocalin-type is responsible for the production of PGD2 in the brain. Lipocalin-type PGD2 synthase (PTGDS), also designated as β-trace, is produced in the leptomeninges and the choroid plexus [33, 412], and is abundant in the cerebrospinal fluid (CSF). The suggestion of altered PGD2 in schizophrenia makes *PTGDS* a good candidate gene in schizophrenia, although it is unknown whether schizophrenic patients display altered prostaglandin signaling inside the brain. The gene is composed of seven exons located on chromosome 9q34.3 [400], a potential susceptibility region for schizophrenia [180]. In addition, PTGDS presents a distal thyroid hormone response element (TRE) [401] and several indications suggest the involvement of genes whose expression is regulated by thyroid hormones in schizophrenia [293, 125]. In accordance, altered gene expression might explain the decreased levels of PTGDS described in the CSF of schizophrenic patients [139, 137, 138].

To test whether genetic variants in the PTGDS increase susceptibility to schizophrenia, we screened all exons, exon-intron boundaries and the promoter of the gene. In order to determine whether the identified variants predispose individuals to develop schizophrenia, association studies were per-

formed using two case-control samples. To overcome the possible existence of population stratification in those samples, we also performed a family-based association study on an independent parents-offspring sample.

4.2 Materials and methods

4.2.1 Samples

Two case-control samples were used: one from Portugal and one from Brazil. The Portuguese sample consisted of 244 cases (175 males and 69 females) and 210 unrelated controls (131 males and 79 females) from northern and central Portugal. The Brazilian sample consisted of 69 cases and 85 controls, all unrelated males from Southern Brazil (Porto Alegre). A third, independent sample consisting of 73 patients (48 males and 25 females) and their parents from the Azores Islands, was used for family-based association analysis. All subjects were of European ancestry, based on the individuals' report of their parents' ethnicity. All participants gave informed consent for genetic studies, and ethic committees of the institutions involved approved the study.

All patients from Portugal-mainland and Azorean Islands, as well as 45% of the Portugal mainland controls and 67% of the Azorean parents were evaluated using the Diagnostic Interview for Genetic Studies (DIGS) [283], a semi-structured interview that assesses the criteria for schizophrenia and other psychiatric diseases. The diagnosis was made based on the Diagnostic and Statistical Manual of Mental Disorders, review of the third edition (DSM-IIIR) [11]. All Brazilian patients were classified using the Operational Checklist for Psychotic Disorders (OPCRIT) [237]. The Brazilian controls (blood donors) as well as the remaining Portuguese controls (students and tissue donors) and Azorean parents were collected without specific psychiatric evaluation (only with the identification of healthy status).

4.2.2 Polymorphism screening and genotyping

The human PTGDS DNA sequence was retrieved from the NCBI GenBank (http://www.ncbi.nlm.nih.gov/Genbank/), with the accession number NT_024000.

Primers for PCRs were designed using the software Primer3 [319].

Using Single Strand Conformational Polymorphism (SSCP) analysis, a subsample of 60 patients from the Portuguese mainland was used for screening DNA variants in the promoter, all seven exons, and intronic sequences adjacent to exons in the *PTGDS* gene. The genotyping was done using a chip-based MALDI-TOF mass spectrometry platform (SEQUENOM, inc., San Diego, CA). We followed the same SSCP and MALDI-TOF approach previously described in detail by some of the authors [320]. The sequences of the PCR primers used are available upon request.

4.2.3 Statistical analysis

Student's *t*-test was used to compare age distributions between patients and controls. Hardy-Weinberg equilibrium and gender distribution were assessed using Chi-square (χ^2) test.

Linkage disequilibrium (LD) between pairs of *loci* was evaluated by r^2. The r^2 measure represents the statistical correlation between two sites, and takes the value of 1 if only two haplotypes are present. There is a simple inverse relationship between r^2 and the sample size required to detect association between susceptibility *loci* and single nucleotide polymorphisms (SNPs) [387]. The r^2 values were computed using the ldmax program from the GOLD software package [2].

Haplotypes were reconstructed using PHASE, version 2.1 [352].

Global genotypic and haplotype distributions ($2 \times N$ contingency tables) were compared between patients and controls by χ^2 test. Allele and specific genotype and haplotype frequencies (2×2 contingency tables) were compared between these two groups by Fisher's exact test. Fisher's exact test is the usual choice for testing the significance in association studies and it is believed to be the most reliable test when one or more of the expected numbers in the 2×2 table is less than five, and when the overall sample size is small [177]. χ^2 test, Fisher's exact test, and *P*-values were computed by InStat 3.06 (GraphPad Software).

Transmission disequilibrium test (TDT) was applied to analyze the results of the family-based association study (Azorean trios sample) using TRANS-

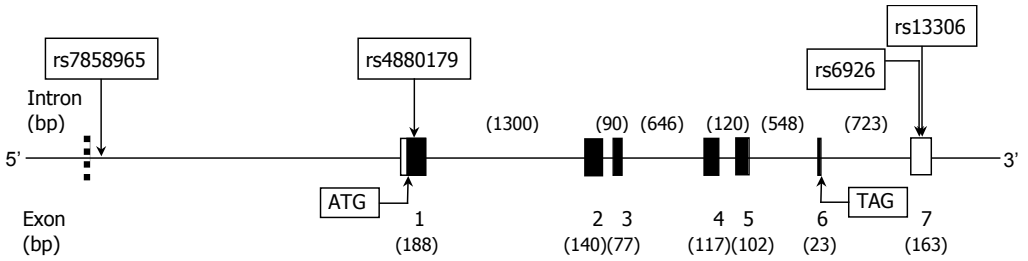

Figure 4.1: Genomic structure of the human *PTGDS* gene and location of the four SNPs detected. Black boxes represent protein-coding regions, white boxes represent untranslated regions, and the dashed line represents the thyroid hormone response element (TRE). Locations of initiation (ATG) and stop (TAG) codons and sizes of introns and exons are also shown. The rs numbers identifying the SNPs are in accordance with the dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/).

MIT, version 2.5.4 [67]. This program computes both global *P*-values, which estimate the significance of transmission distortion for all the haplotypes tested, as well as individual *P*-values for the significance of transmission distortion of specific haplotypes. All tests were two-tailed and results were considered to be significant if the *P*-value was less than 5%.

4.3 Results

Using SSCP, we screened the *PTGDS* gene in 60 schizophrenic patients and identified four SNPs that have been previously recorded in the NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/). The rs7858965 SNP is located in the promoter region (80bp downstream from the thyroid hormone response element), rs4880179 is a synonymous change on exon 1, and rs6926 and rs13306 lie in the non-coding exon 7 (Figure 4.1). Applying r^2 analysis, which measures the correlation between alleles, to genotype data generated by the SSCP screening (60 schizophrenic patients from Portugal mainland) we estimated that the rs7858965 and rs4880179 variants were in complete LD (r^2=1.0 and *P*-value$<$0.001). Due to the complete LD, only one of these variants (rs4880179) was genotyped in the complete samples described above.

Analysis of gender distribution in the Portuguese mainland sample showed

Table 4.1: PTGDS SNPs genotyped in case-control samples. Represented are the frequencies of the minor allele.

SNP ID	Alleles	Sample	Frequency		HWE
	major>minor	origin	Cases	Controls	(overall)
rs4880179	G>A	Portugal mainland	0.037	0.044	0.731
		Brazil	0.015	0.031	0.769
rs6926	C>A	Portugal mainland	0.267	0.278	0.608
		Brazil	0.313	0.280	0.460
rs13306	A>G	Portugal mainland	0.035	0.043	0.684
		Brazil	0.014	0.035	0.741

that males are significantly (P-value=0.034) more frequent in cases (71.7%) than in controls (62.4%). Age distribution was well matched between patients and controls for both Portuguese mainland and Brazilian samples (P-value$>$0.4 for both samples). The same was true after the Portuguese mainland sample was stratified by gender (P-value=0.232 for males and P-value= 0.443 for females).

None of the three SNPs' genotype distribution showed any significant deviation from Hardy-Weinberg equilibrium, either in controls, patients, parents or overall groups.

Genotype and allele frequencies at the three SNPs analysed did not differ significantly between the schizophrenic patients and the controls either in the Portuguese mainland or in the Brazilian samples (Table 4.1).

Haplotypes were estimated by PHASE from the genotypic data. Two major haplotypes, among the 6 estimated, showed a frequency$>$5% (Table 4.2). They accounted for more than 95% of the estimated haplotypes in patients and controls, both in the Portuguese-mainland and Brazilian samples. There were fewer haplotypes than can be expected from three SNPs (23=8), indicating existence of linkage disequilibrium across the alleles analysed. Based on r^2, the SNPs rs4880179 and rs13306 are tightly correlated (r^2 $>$0.75 and P-value$<$0.001 for all samples). These two SNPs are poorly correlated with the rs6926 SNP (r^2 approximately 0.1).

There were no significant differences in haplotype distributions (Table 4.2)

Table 4.2: Estimated haplotype frequencies and significance of association in the case control samples.

				Sample size (%)		
Haplotypes[a]	rs4880179	rs6926	rs13306	Cases	Controls	*P*-value[b]
Portugal-mainland				n=480	n=410	
1	G	C	A	351 (73.1)	296 (72.2)	0.763
2	G	A	A	111 (23.1)	97 (23.7)	0.874
3	A	A	G	17 (3.5)	17 (4.1)	0.727
Global						0.876
Brazil				n=130	n=160	
1	G	C	A	89 (68.5)	114 (71.3)	0.609
2	G	A	A	39 (30.0)	41 (25.6)	0.430
3	A	A	G	1 (0.8)	5 (3.1)	0.229
Global						0.287

[a]Haplotypes formed by the 3 SNPs genotyped were reconstructed with the Phase program. Haplotypes with an estimated probability less than 1% are not reported.

[b]*P*-value was assessed using Fisher's exact test with the exception of the global *P*-value that was obtained by χ^2 test with 2df. Fisher's exact tests were derived from a series of simple 2×2 tables based on the frequency of each haplotype versus all others combined between the case and control groups.

between cases and controls in either the Portuguese mainland or the Brazilian sample. This was also true after gender stratification of the Portuguese mainland sample.

TDT results were concordant with the results of case-control association studies since no excess of transmission was observed from parents to offspring in the 73 parents-offspring sample from the Azorean Islands (Table 4.3).

4.4 Discussion

Our results do not support the hypothesis that inherited variants in the *PTGDS* gene are involved in the etiopathophysiology of schizophrenia. By screening all exons, intron-exon boundaries and most of the promoter region, we found four polymorphisms already identified in the dbSNP database and none of the variants tested is associated with schizophrenia.

Table 4.3: Estimated haplotype frequencies, and *P*-values for haplotype transmission calculated with the TRANSMIT program.

				Haplotype		
Haplotype	rs4880179	rs6926	rs13306	Observed	Expected	*P*-value
1	G	C	A	101	101.48	0.887
2	G	A	A	38	37.52	0.888
3	A	A	G	5	4.98	0.909
4	A	A	A	0	0.50	0.262
5	A	C	A	0	0.52	0.259
6	G	A	G	2	1.00	0.213
Global						0.550

However, we did not exclude that the studied DNA variants could be related to a particular symptom or feature of schizophrenia. In fact, Miwa studied hypertensive patients and found an association of the rs6926 AA genotype of the *PTGDS* gene with an increase in cholesterol levels [254]. Interestingly, cholesterol levels are higher in schizophrenics when compared with controls [173] independent of medication, giving additional evidence of lipid metabolism dysregulation in these patients. The Miwa study has been conducted in a Japanese population and we know from the HapMap project (http://www.hapmap.org/) that the frequency of the rs6926 AA genotype is somewhat different in the Japanese (0.023) and European (0.083) populations. Still, if the high levels of cholesterol found in schizophrenic patients have a genetic basis and are not consequence of their medication, poor diet, or of a more sedentary life we might expect to find a similar association of PTGDS genotypes in our samples.

Since altered expression of genes could result not only from DNA variants in the regulatory regions of genes but also from inadequate supply of modulators of transcription activity [280], the fact that we failed to detect association of the studied DNA variants with schizophrenia does not exclude that the PTGDS expression levels could be altered in the brains of patients. It has been shown that thyroid hormones [109, 110] and retinoids [175] modulate the expression of the *PTGDS* gene. Abnormal availability of thyroid hormones and retinoids

has been suggested to be involved in the etiology of schizophrenia [293, 125], and such abnormalities could explain the low CSF level of PTGDS reported in these patients [139, 137, 138].

Consequences of altered PTGDS levels are not known, but given the important roles proposed for the PTGDS enzyme and its product, PGD2, the altered expression of the *PTGDS* gene may contribute to the multitude of biological abnormalities observed in the schizophrenia pathology. For instance, studies in rodents have shown that PTGDS plays an important role in the regulation of sleep-wake cycles [359, 231] and in sensitivity to tactile pain [89], which are both known to be altered in schizophrenic patients [90, 232].

Interestingly, the relation of PTGDS with thyroid hormones and retinoids is reciprocal. On the one hand, these latter two control the expression of the *PTGDS* gene. On the other hand, since PTGDS has been described as a carrier for thyroid hormones and retinoids [362, 31], abnormal levels of PTGDS can eventually disturb their availability for modulation of the expression of a large number of genes, including PTGDS itself. In this way, PTGDS could contribute to the deleterious effects of altered thyroid hormones and retinoids in the central nervous system.

In conclusion, although we failed to detect involvement of *PTGDS* gene variants in the genetic susceptibility to schizophrenia in the populations studied, it seems worthwhile to explore the effect of altered PTGDS levels as a consequence of the modulation of transcription and its relationship with the disease pathology.

5

Transthyretin and schizophrenia

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Abstract

It has been proposed that schizophrenia results from an environmental insult in genetically predisposed individuals. Environmental factors capable of modulating transcriptional activity and their carriers could link the genetic and environmental components of schizophrenia. Among these is transthyretin (TTR), a major carrier of thyroid hormones and retinol-binding protein (RBP). Retinoids and thyroid hormones regulate the expression of several genes, both during development and in the adult brain. Decreased TTR levels have been reported in the cerebrospinal fluid of patients with depression and Alzheimer's disease, and the absence of TTR influences behavior in mice. DNA variants capable of altering TTR ability to carry its ligands, either due to reduced transcription of the gene or to structural modifications of the protein, may influence development of the central nervous system and behavior. In the present study we searched for variants in the regulatory and coding regions of the *TTR* gene, and measured circulating levels of TTR and RBP. We found a novel single nucleotide polymorphism (SNP), ss46566417, 18bp upstream of exon 4. Neither this SNP nor the previously described rs1800458 were found associated with schizophrenia. In addition, serum TTR and RBP levels did not differ between mentally healthy and schizophrenic individuals. In conclusion, our data does not support an involvement of the *TTR* gene in the pathophysiology of schizophrenia.

5.1 Introduction

Schizophrenia is a complex neurodevelopment disorder [28]. Epidemiological studies indicate an increased risk for developing schizophrenia in relatives of probands with the disease [126], suggesting a genetic predisposition. However, monozygotic twins are frequently phenotypically discordant, which implies that environmental factors must play a role in the disease etiology [372].

Thyroid hormones and retinoids are among the environmental factors proposed to be altered in schizophrenia [125, 293]. It is widely recognized that

thyroid hormones and retinoids are essential for the normal development of the central nervous system, and that lack of adequate levels during pregnancy leads to several neurological defects [195, 217, 267, 268, 406]. Thyroid hormones and retinoids act as modulators of the expression of several genes through the binding of retinoic acid and triiodothronine to the corresponding nuclear receptors. Interestingly, evidence of altered retinoid and thyroid hormone metabolisms in schizophrenic patients is arising from studies in postmortem brains, in which RAR and myelin-basic protein, genes whose expression is regulated by thyroid hormones and retinoids, were found to have altered expression [134, 309].

By influencing ligand availability, carriers of thyroid hormones and retinoids indirectly regulate the transcription of several genes. Among the modulators of thyroid hormone and retinoid availability is transthyretin (TTR), a major carrier of thyroxine and retinol (through association with RBP], both in serum and in cerebrospinal fluid (CSF) [292]. TTR has been implicated in behavior: decreased TTR CSF levels were found in patient) with depression [357] and with Alzheimer's disease [337]. Whether this decrease is the result or a consequence of the disease or whether it is caused by medication is still unclear. Supporting that both processes might be implicated in behavior are the observations that TTR-null mice [292] present increased motor activity in behavior tests that address anxiety-like and depression-like behaviors [349], and that clozapine treatment induces TTR expression in the brain [59].

The *TTR* gene is a single copy gene, on 18q12.1, composed of four exons [373] that encodes a 14 kDa subunit which assembles as a tetramer [32]. Whereas plasma TTR originates primarily from the liver, CSF TTR is mainly produced and secreted from the choroid plexus, where it represents about 20% of the total protein synthesis [9]. The observation that homologues of the TTR protein can be found in a wide range of species [94] and that its synthesis starts early during embryonic development [197], suggests a relevant role for TTR in development.

These observations, prompted us to investigate the *TTR* gene as a candidate gene in schizophrenia. In the present study we searched for variants in the regulatory and coding regions of the *TTR* gene, and association be-

tween the polymorphisms detected and schizophrenia were analyzed in three different samples. We also investigated serum TTR and RBP levels in schizophrenic and in mentally healthy individuals.

5.2 Materials and methods

5.2.1 Samples

Two case-control samples were used: one from Portugal-mainland and a second from Brazil. A total of 244 unrelated schizophrenic patients (175 males and 69 females) and 210 controls (131 males and 79 females) were recruited from the north and center of Portugal-mainland. The Brazilian sample consisted of 69 cases and 85 controls, all unrelated males living in the area of Porto Alegre. A third, independent sample composed of 73 patients (47 males and 26 females) and their parents from the Azorean Islands (Portugal), was used for family-based association analysis. All subjects were of European ancestry. All participants gave informed consent for genetic studies and ethic committees of the institutions involved approved the study.

All patients from Portugal-mainland and Azorean Islands, as well as 45% of Portugal-mainland controls and 67% of the Azorean parents were evaluated using the Diagnostic Interview for Genetic Studies (DIGS) [283], a semi-structured interview that assesses the criteria for schizophrenia and other psychiatric diseases. The diagnosis was made based on the Diagnostic and Statistical Manual of Mental Disorders, review of the third edition (DSM-IIIR) [11]. All Brazilian patients were classified using the Operational Checklist for Psychotic Disorders (OPCRIT) [237]. No psychological assessment interviews have been conducted in the remaining Portuguese mainland controls (students and organ donors) or Azorean parents, or in any of the Brazilian controls (blood donors).

5.2.2 Polymorphism screening

Using single strand conformational polymorphism (SSCP) analysis, a subsample of 60 patients from the Portuguese mainland was used for screening

variants in all four exons and adjacent splicing sites, as well as the promoter and the 3´ untranslated regions. For this analysis the PCR products were subjected to electrophoresis on a non-denaturing polyacrylamide gel under two temperature conditions: 4ºC and 25ºC. After completion of the electrophoresis, band patterns were visualized with silver staining, using standard protocols. PCR products from subjects displaying altered band patterns in the SSCP analyses were sequenced in both directions. The sequencing reactions were performed using the BigDye Sequencing Kit 3.1 and run on the 3700 sequencer, both from Applied Biosystems (Foster City, CA).

The TTR sequence was obtained from the GenBank Data Libraries (accession no. M11844) and the Primer3 program [319] was used for primer design. Primer sequences are available upon request.

5.2.3 SNP genotyping

Two single nucleotide polymorphisms (SNP) were detected: rs1800458 on exon 2 and ss46566417 on intron 3 (Figure 5.1). These two SNPs were further typed for association in the complete samples described above.

Detection of rs1800458 and ss46566417 was based upon analysis of primer extension products generated from previously amplified genomic DNA, using a chip-based MALDI-TOF mass spectrometry platform (SEQUENOM Inc., San Diego, CA). PCR primers with a universal (10 bp) sequence at the 5´ end (5´- ACGTTGGATG-3´) and the extension primer were created using the MassARRAY Assay Design 2.0 software (SEQUENOM), following manufacturer's instructions. For the MALDI-TOF analysis, aliquots of the samples were spotted onto a SpectroCHIP (SEQUENOM). Mass spectra were generated by the MassARRAY spectrometer (SEQUENOM). Genotypes were automatically determined using the SpectroTYPER software (SEQUENOM). In some cases genotyping of rs1800458 was repeated using PCR-based restriction fragment length polymorphism assay. The same PCR product used for the SSCP analysis was digested with the endonuclease Cfr 10I (Fermentas, Vilnius, Lithuania). The digested reactions were resolved by electrophoresis on 3% agarose gels.

5.2.4 Measurement of TTR and RBP serum levels

Twenty six schizophrenic patients (16 males and 10 females) and 54 controls (25 males and 29 females) from Portugal-mainland as well as 27 schizophrenic patients (15 males and 12 females) from the Azorean Islands were used to measure the serum levels of TTR, RBP, and albumin. Serum TTR, RBP, and albumin were determined by radial-immunodiffusion in accordance to the manufacturer's instructions (The Binding Site Limited, Birmingham, UK).

5.2.5 Statistical analysis

The genotype and allele frequencies were compared between cases and controls using a standard χ^2 test, calculated by SPSS (version 13.0). Hardy-Weinberg Equilibrium (HWE) was assessed in the same way.

To analyze the results of the family-based association study (Azorean sample), a transmission disequilibrium test (TDT) was performed, using TRANSMIT version 2.5.4 [67].

Student's t-test was used to compare ages and protein serum levels between patients and controls. Association between serum TTR, RBP and albumin levels and their correlation with age was analyzed by linear regression. These last two analyses were also performed with SPSS. For all tests, the results were considered significant when the *P*-value was less then 5%.

5.3 Results

5.3.1 Association study

In the 60 patients screened for variants we detected the presence of two SNPs: the previously described rs1800458 on exon 2 and the novel ss46566417, 18bp upstream from exon 4 (Figure 5.1). The novel SNP has been submitted to the NCBI SNP database (http://www.ncbi.nlm.nih.gov/SNP/).

HWE was verified for all tested samples, showing no deviation for either SNP analyzed (Table 5.1). Table 5.1 summarizes the genotyping results for

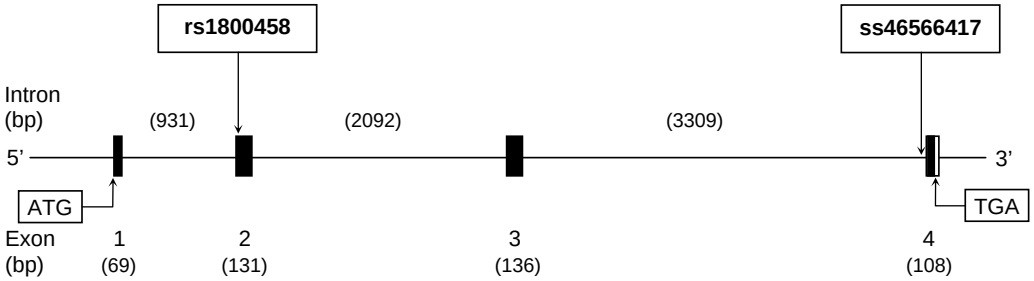

Figure 5.1: Genomic structure of human *TTR* gene and locations of the two SNPs analyzed. Black boxes represent protein-coding regions, white boxes represent untranslated regions. Locations of initiation (ATG) and stop (TGA) codons and sizes of introns and exons are also provided. The rs and ss numbers identify the SNPs according with the dbSNP database of NCBI (http://www.ncbi.nlm.nih.gov/SNP/).

Table 5.1: Case-Control analyses for the two SNPs studied

Origin	SNP (phenotype)	Genotype (frequencies)			*P*-value	HWE
Portugal	rs1800458	GG	GA	AA		
mainland	Cases (n=163)	0.914	0.086	0.000	0.496	0.567
	Controls (n=161)	0.888	0.106	0.006		0.533
	ss46566417	GG	GC	CC		
	Cases (n=244)	0.906	0.094	0.000	0.513	0.440
	Controls (n=209)	0.890	0.105	0.005		0.691
Brazil	rs1800458	GG	GA	AA		
	Cases (n=69)	0.855	0.145	0.000	0.617	0.516
	Controls (n=85)	0.882	0.118	0.000		0.564
	ss46566417	GG	GC	CC		
	Cases (n=69)	0.928	0.072	0.000	0.964	0.755
	Controls (n=85)	0.929	0.071	0.000		0.736

Represented are the genotype frequencies, *P*-values and HWE values.

the two SNPs. No significant differences between cases and controls were detected in genotype or allele frequencies, either in the Portuguese mainland or in the Brazilian samples. The lack of association was confirmed in the Azorean sample, since the TDT revealed no excess of transmission from parents to offspring (Table 5.2).

Table 5.2: Individual SNP analysis by TRANSMIT.

SNPs	major>minor allele (frequency[a])	χ^2 (df=1)	*P*-value
rs1800458	G>A (0.034)	0.517	0.472
ss46566417	G>C (0.045)	0.077	0.782

[a]Frequency of the minor allele, produced by the TRANSMIT program.

5.3.2 TTR, RBP and albumin serum levels

Serum levels of TTR, RBP and albumin did not differ between schizophrenic and mentally healthy individuals, whether male or female. There were no statistical differences of TTR, RBP or albumin serum levels between the analyzed Portuguese mainland and Azorean patients. Therefore these values are presented together in Table 5.3. Distribution of age in the sample used was not well matched between patients and controls (Table 5.3). However, within this age-range, age did not influence the levels of TTR, RBP and albumin, as verified by linear regression analysis (*P*-value=0.176 for TTR, *P*-value=0.330 for RBP and *P*-value=0.736 for albumin). As expected, TTR and RBP levels were positively correlated, when analyzed by linear regression (*P*-value$<$0.0005).

5.4 Discussion

In the present study we describe a novel TTR polymorphism, ss46566417 with an allele frequency of 0.048 in Caucasians [0.052 (47/906) in Portuguese from mainland, 0.048 (14/292) in Azorean, and 0.036 (11/308) in Brazilian]. This novel polymorphism does not change the amino-acid sequence of the protein, since it is localized on intron 3, but might interfere with transcription given its close proximity to a splicing site. We also detected the SNP rs1800458 at an allele frequency (0.059) similar to that previously described in the Caucasian population [172]. Neither of these two SNPs was found associated with schizophrenia. While the present study, as many others, failed to identify genetic variants that predispose to schizophrenia, recent studies in brains from schizophrenic patients have shown altered expression of several

Table 5.3: TTR, RBP and albumin serum levels in patients and in controls.

	Males		*P*-value	Females		*P*-value
	Cases	Controls		Cases	Controls	
N	31	25		22	29	
Age	36.6±15.0	29.7±6.8	0.028	42.2±11.4	31.6±9.9	0.001
TTR (mg/L)	296.9±49.4	295.4±34.6	0.898	277.0±51.1	284.3±49.5	0.610
RBP (mg/L)	67.8±16.3	65.4±9.9	0.495	64.9±13.9	61.8±13.4	0.427
Albumin (mg/L)	42832±5585	43940±5789	0.473	41027±4385	41183±4900	0.906

Data represented as means±SD.

genes [134, 252]. Such alterations might result from variants in the regulatory regions of the genes but also from inadequate availability of transcription modulators such as hormones and vitamins [293]. For these reasons, and even though we failed to detect association of the TTR polymorphisms with schizophrenia, in these two populations, we investigated the circulating levels of TTR and also of RBP. Serum levels of RBP did not differ between schizophrenic and mentally healthy individuals. Being the only plasma carrier for retinol, RBP is measured as a surrogate marker of retinol in the circulation [70]. Therefore, our data suggest that serum retinoid status is not altered in patients diagnosed with schizophrenia, since retinol is the most common form of retinoids in circulation. This is, however, in contrast with another study in which the serum levels of RBP and other inflammatory acute phase proteins such as albumin were found decreased in the serum of Chinese schizophrenic patients from Singapore [409]. Given that our patient sample showed normal albumin serum levels, and since RBP is a useful marker of protein malnutrition [70] and of inflammatory acute phase response [103], it is possible that the values observed in that study might reflect a particular nutritional or inflammatory state in the patients. While we found no changes in RBP circulating levels in our patient population, search for RBP variants should be investigated since these could impair the ability to carry retinol and/or to bind to TTR.

Ours is the first report on serum levels of TTR and we found no differences between schizophrenic and control individuals. It should be noted that serum TTR levels do not necessarily reflect CSF levels since the first is mainly derived form the liver while the latter is primarily synthesized and secreted from

the choroid plexus [9]. In fact, TTR expression has been described as differentially regulated in the liver and in the choroid plexus [80]; probably due to the presence of tissue specific transcription factors. Recent interest in the possible decrease of CSF TTR during aging has been raised mainly in the context of Alzheimer's disease, since TTR is also able to bind the Alzheimer β peptide [54]. It would therefore be interesting to measure CSF TTR levels in patients, where available, to extend a single study in which CSF TTR levels were reported normal in patients with schizophrenia [34]. All together the data obtained to date does not support the involvement of TTR in the pathophysiology of schizophrenia. However, it is important to investigate whether TTR polymorphisms are related to any particular symptom or response to medication, or whether the levels of TTR ligands, rather then TTR itself, are involved in the etiology of schizophrenia.

6

Neurogranin and schizophrenia

This chapter is currently in press as: Ruano D, Aulchenko YS, Macedo A, Soares MJ, Valente J, Azevedo MH, Hutz MH, Gama CS, Lobato MI, Belmonte-de-Abreu P, Goodman AB, Pato C, Heutink P, Palha JA, Association of the gene encoding neurogranin with schizophrenia in males, J Psychiatr Res (2006), doi:10.1016/j.jpsychires.2006.10.008.

Abstract

The *neurogranin* (*NRGN*) gene produces a postsynaptic brain-specific protein that regulates calmodulin-Ca^{2+} availability in neurons. Acting downstream of the NMDA-receptor and upstream of calcineurin and other proteins implicated in schizophrenia, NRGN is a good candidate for association studies in schizophrenia. NRGN expression is regulated during development and is modulated by thyroid hormones and retinoids, molecules essential for the proper development of the central nervous system. Given the genetic complexity of schizophrenia and the potential genetic heterogeneity in different populations, we studied a possible association of NRGN with schizophrenia in 73 Azorean proband-parent triads and in two independent case-control samples from the Portuguese mainland (244 schizophrenic and 210 controls) and Brazil (69 schizophrenic and 85 mentally healthy individuals). Genotype distribution showed association of the rs7113041 SNP with schizophrenia in males of Portuguese origin, which was confirmed by the analysis of the proband-parent triads. This evidence, implicating NRGN in schizophrenia, introduces another player into the glutamatergic hypothesis of schizophrenia.

6.1 Introduction

Family, twin, and adoption studies clearly indicate a genetic contribution to the etiology of schizophrenia [238, 239]. The frequent discordance of monozygotic twins in developing schizophrenia, suggests that environmental factors are also involved in disease manifestation [126]. The disease is proposed to result from a neurodevelopmental impairment occurring during gestation that is caused by a combination of genetic and environmental factors [372]. Age of onset ranges from mid to late adolescence through early adulthood, affecting males more often and more severely than females [279, 10].

Despite the family aggregation of schizophrenia, linkage studies have failed to identify a major risk chromosomal region [301], which suggests the involve-

ment of multiple genes each conferring a minor increase in disorder susceptibility [347]. The idea of a polygenic origin of the disease is reinforced by studies in schizophrenia postmortem brains revealing altered expression of several genes and proteins [134, 252]. Altered expression of genes could result either from polymorphisms in their regulatory regions, e.g. the promoter, or from inadequate supply of modulators of transcription activity [280]. Accordingly, it has been suggested that modulators, such as hormones and vitamins, are a possible link between the genetic and environmental components of the complex schizophrenia disorder [125, 293].

Thyroid hormones and retinoids are essential for the normal mammalian brain development [221, 268]. They regulate processes such as differentiation of the cerebellum, axonal migration and myelination [317], and the control of lateralization and symmetry of the embryo [383, 382]; processes that have been implicated in schizophrenia [293]. Their mechanism of action is mainly through the regulation of transcription of genes including those encoding myelin basic protein [317], dopamine receptor D2 [327] and the receptor for neuregulin1, ERBB4 [285]; all genes previously implicated in schizophrenia.

Among the genes whose expression is regulated by thyroid hormones and retinoids is the *neurogranin* (*NRGN*) gene [93, 130, 161, 165] that encodes a postsynaptic brain-specific protein. The human *NRGN* gene, localized on chromosome 11q24.2 [228], consists of four exons and three introns. The 78-amino-acid protein is encoded by part of exon 1 and by exon 2 [228] and a thyroid hormone response element (TRE) has been identified in intron 1 [227]. Information on the function of NRGN stems from studies in rodents. NRGN accumulates in dendritic spines of specific neurons within the cerebral cortex, hippocampus, striatum, and amygdala [276, 394]. During development, NRGN expression is regulated by maternal thyroid hormones [81] and its highest expression is coincident with the development period characterized by rapid dendritic growth and formation of the majority of the cortical synapses [165]. Of particular relevance is the fact that NRGN binds calmodulin (CaM) with high affinity [108, 300, 303], buffering postsynaptic CaM levels and modulating the concentration of Ca^{2+} in neurons [157]. Phosphorylation by protein kinase C (PKC) or oxidation by nitric oxide decreases CaM-binding affinity [156, 211].

Glutamate stimulation of N-methyl-D-aspartate (NMDA) receptors results in Ca^{2+} influx to the neuron and in NRGN oxidation [211]. These induce dissociation of the NRGN-CaM complex and stimulate the phosphorylation of NRGN by PKC [318], which prevents the re-binding of NRGN and CaM. As a CaM reservoir, NRGN regulates the release of CaM and the activities of downstream CaM-Ca^{2+}-dependent enzymes that play an important role in the neuroplasticity mechanisms of learning and memory [157, 291]. Therefore, altered NRGN activity could mediate the effects of NMDA receptor hypofunction suggested by several studies to be implicated in the pathophysiology of schizophrenia [368]. These observations prompted us to investigate *NRGN* as a candidate gene in schizophrenia.

Case-control studies compare allele and genotype distributions between affected and unaffected individuals. When a significant result is obtained, it may be due to linkage between the marker and the disease but can as well reflect population stratification. Family-based association analysis compares the allele and genotype frequencies between cases and their parents to explore if a specific marker is transmitted to a greater degree than expected under Mendelian inheritance. This analysis is robust against possible population stratification. Therefore, we decided to conduct the association study of NRGN using both case-control samples (from Portugal-mainland and Brazil) and family-based samples (from Azorean Islands).

6.2 Materials and methods

6.2.1 Samples

Two case-control samples were used: one from Portugal and one from Brazil. The Portuguese sample consisted of 244 cases (175 males and 69 females) and 210 unrelated controls (131 males and 79 females), recruited from the north and center of the Portuguese mainland. Patients with clinical diagnosis of schizophrenia were systematically drawn from both the inpatient and outpatient clinic at the University Hospital of Coimbra. The Brazilian sample consisted of 69 cases and 85 controls, all unrelated males living in the area

of Porto Alegre. All Brazilian patients were under outpatient care. A third, independent sample consisting of 73 patients (48 males and 25 females) and their parents from the Azorean Islands, was used for family-based association analysis. Based on the individuals' report of their parents' ethnicity, on family history, and on the observation of skin, facial and hair characteristics, all subjects were classified as Caucasians. The Porto Alegre region, in the southern part of Brazil, is considered to have 92% of European ancestry, mostly Portuguese, Spanish, German and Italian [105, 331]. All participants gave informed consent for genetic studies, and ethic committees of the institutions involved approved the study.

All patients from Portugal-mainland and Azorean Islands, as well as 45% of Portugal-mainland controls and 67% of the Azorean parents were evaluated using the Portuguese version of the Diagnostic Interview for Genetic Studies (DIGS) [283], a semi-structured interview that assesses the criteria for schizophrenia and other psychiatric diseases. The diagnosis was made based on the Diagnostic and Statistical Manual of Mental Disorders, review of the third edition (DSM-IIIR) [11]. All Brazilian patients were classified using the Operational Checklist for Psychotic Disorders (OPCRIT) [21, 237]. No psychological assessment interviews have been conducted in the remaining Portuguese mainland controls (students and tissue donors) or Azorean parents or in any of the Brazilian controls (blood donors).

For 97% of the Portuguese mainland, 44% of the Azorean and 87% of the Brazilian patients, information on age of onset, based on the OPCRIT criteria, was available. Specifically, for males, it was 22 ± 6 (n=171), 20 ± 4 (n=21), and 20 ± 5 (n=60), respectively; and for females, 22 ± 6 (n=66) and 26 ± 7 (n=11). Please note that the Brazilian sample is all males.

Blood was drawn from all subjects and DNA extracted using standard salting-out procedures.

6.2.2 Polymorphism screening

Using Single Strand Conformational Polymorphism (SSCP) analysis, a subsample of 60 patients from the Portuguese mainland was used for screening

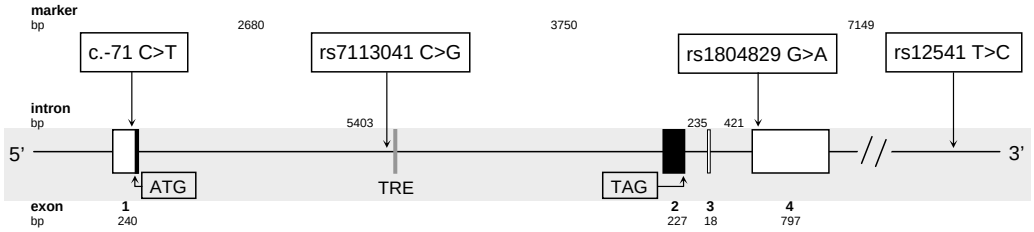

Figure 6.1: Genomic structure of the human *NRGN* gene and positions of the three SNPs tested and of the mutation found. Black boxes represent protein-coding regions, white boxes represent untranslated regions, and the grey line represents the thyroid hormone response element (TRE). Locations of initiation (ATG) and stop (TAG) codons, sizes of introns and exons, and physical distances between markers are also shown. The rs numbers identifying the SNPs are in accordance with the dbSNP database

DNA variants in the four exons and adjacent splicing sites, as well as in the thyroid hormone response element (TRE) on intron 1 of the *NRGN* gene. For this analysis the PCR products were subjected to electrophoresis on a non-denaturing polyacrylamide gel under two temperature conditions: 4°C and 25°C. After completion of the electrophoresis, PCR products were visualized with silver staining. PCR products from subjects displaying altered band patterns in the SSCP analyses were sequenced in both directions. The sequencing reactions were performed using BigDye Sequencing Kit 3.1 and run on a 3700 sequencer, both from Applied Biosystems (Foster City, CA).

The NRGN sequence was obtained from the GenBank Data Libraries (accession no. AJ317956), and the Primer3 program [319] was used for primer design. Primer sequences are available upon request.

6.2.3 SNP genotyping

Three previously described single nucleotide polymorphisms (SNPs) were genotyped: rs7113041 in intron 1, rs1804829 in exon 4, and rs12541 localized downstream of the exon 4 (Figure 6.1).

Specifically, rs12541 was genotyped using a PCR-based restriction fragment length polymorphism (RFLP) assay. The PCR product was submitted to digestion using Bfm I endonuclease (Fermentas, Vilnius, Lithuania). The digested reactions were resolved by electrophoresis on agarose gel.

Detection of SNPs rs7113041 and rs1804829 was based upon analysis of primer extension products generated from previously amplified genomic DNA, using a chip-based MALDI-TOF mass spectrometry platform (Sequenom Inc., San Diego, CA). PCR primers with a universal (10 bp) sequence at the 5´ end (5´-ACGTTGGATG-3´) and the extension primers were created using the MassARRAY Assay Design 2.0 software (Sequenom), following manufacturer's instructions. For the MALDI-TOF analysis, aliquots of the samples were spotted onto a SpectroCHIP (Sequenom). Mass spectra were generated by the MassARRAY spectrometer (Sequenom). Genotypes were automatically determined using the SpectroTYPER software (Sequenom).

6.2.4 Statistical analysis

Student's t-test was used to compare age distributions between patients and controls. Hardy-Weinberg equilibrium and gender distribution were assessed using Chi-square (χ^2) test.

Linkage disequilibrium (LD) between pairs of *loci* was evaluated by r^2. The r^2 measure represents the statistical correlation between two sites and takes the value of 1 if only two haplotypes are present. There is a simple inverse relationship between r^2 and the sample size required to detect association between susceptibility *loci* and SNPs [387]. The r^2 values were computed using the ldmax program from the GOLD software package [2].

Global genotypic distributions ($2 \times N$ contingency tables) were compared between patients and controls by χ^2 test. Allele and specific genotype frequencies (2×2 contingency tables) were compared between these two groups by Fisher's exact test, instead of the χ^2test. Fisher's exact test is the usual choice for testing the significance in association studies and it is believed to be the most reliable test when one or more of the expected numbers in the 2×2 table is less than five, and when the overall sample size is small [177]. Confirmation of the case-control results was done in the family-based sample by evaluating transmission distortions using pedigree disequilibrium test (geno-PDT) [226].

Genotype frequencies among controls from Portugal-mainland and Brazil, and between these two groups and the genotypes made of non-transmitted

alleles in the trios sample were compared using χ^2 test. This was done both for the overall samples and for the male subsamples. The same test was used for comparing genotype frequencies among cases from different origins and in overall and male subsamples. χ^2 test, Fisher's exact test, *P*-value, and Odds Ratios (ORs) and the 95% confidence intervals (CI) were computed by InStat 3.06 (GraphPad Software). All tests were two-tailed and results were considered to be significant if the *P*-value was less than 5%.

6.3 Results

By SSCP, a new single nucleotide variant (c.-71C>T on exon 1) [mutations nomenclature follow recommendation by Antonarakis [15]] and two previously described SNPs (rs7113041 in intron 1 and rs1804829 in exon 4) were identified (Figure 6.1). These SNPs together with a third SNP (rs12541 located downstream the gene) obtained from the NCBI SNP database, were further genotyped in the complete samples described in subsection 2.1. The c.-71C>T variant was found in one heterozygous out of 60 cases screened, and was not used in the association study given its low frequency.

Analysis of gender distribution in the Portuguese mainland sample showed that males have significantly (*P*-value=0.034) higher relative frequency in cases (71.7%) than in controls (62.4%). Distribution of age was well matched between patients and controls for both Portuguese mainland and Brazilian samples (*P*-value>0.4 for both samples). The same was true after the Portuguese mainland sample was stratified by gender (*P*-value=0.232 for males and *P*-value=0.443 for females).

Pairwise LD was calculated between the three SNPs in the two case-control samples and in the 146 unrelated parents (See Table 6.1). We detected a weak but significant LD between rs7113041 and rs12541 in the three samples (r^2 >0.1 and *P*-values<0.001 for all samples). This result is concordant with the HapMap data. The *P*-values obtained for the LD analysis of the SNPs rs7113041 and rs1804829 are not significant (*P*-value>0.05) and thus we can not conclude on linkage disequilibrium between them. In this case, it

Table 6.1: Linkage disequilibrium, measured by r^2, between the polymorphic *loci* for all samples studied.

Combination	Portuguese mainland	Brazil	Azorean parents
rs7113041-rs1804829			
r^2 (*P*-value)	0.005 (0.097)	0.020 (0.096)	0.009 (0.083)
rs7113041-rs12541			
r^2 (*P*-value)	0.135 (0.000)	0.313 (0.000)	0.138 (0.000)
rs1804829-rs12541			
r^2 (*P*-value)	0.011 (0.002)	0.012 (0.307)	0.008 (0.258)

appears that the minor allele of one SNP is in linkage disequilibrium with the major allele of the other SNP, a scenario under which large sample sizes are needed to obtain significance. The SNPs rs1804829 and rs12541 were found not to be in LD in the largest sample (Portuguese-mainland, *P*-value=0.002, r^2=0.011.). However, though on average LD decreases with physical distance, that does not always occur [3].

In view of the gender mismatch in the Portuguese mainland sample, this sample was stratified by gender (note that the Brazilian sample is composed of males only). After gender stratification, we found a significant excess of the CG genotype at the rs7113041 marker in males (Table 6.2). In this case-control sub-sample, the frequency of rs7113041-CG was 43.4% in patients and 30.3% in controls, with this genotype predisposing males to develop the disease (OR=1.764; CI=1.082 to 2.874). In the same way, though reaching marginal significance only (Table 6.2), the Brazilian case-control sample showed an excess of rs7113041 heterozygous in patients (53.0%) when compared with controls (36.7%), with this genotype also increasing the predisposition to the disease (OR=1.947; CI=1.000 to 3.788). In both case-control samples, no differences between cases and controls were found in the genotype distribution of the other two markers, rs1804829 and rs12541, nor in the allelic distribution of any of the three SNPs.

In the sample of the 73 Azorean parents-offspring, no transmission distortion of alleles was found for any of the three SNPs analyzed. However, using

Table 6.2: Genotype distributions at marker rs7113041.

Sample	Total sample		*P*-value[b]	Male		*P*-value[b]	Female		*P*-value[b]
origin	Case	Control		Case	Control		Case	Control	
Portugal mainland									
CC	131 (53.7)	113 (57.4)	0.500	92 (52.6)	76 (62.3)	0.122	39 (56.5)	37 (49.3)	0.408
CG	103 (42.2)	71 (36.0)	0.203	76 (43.4)	37 (30.3)	0.029	27 (39.1)	34 (45.3)	0.502
GG	10 (4.1)	13 (6.6)	0.284	7 (4.0)	9 (7.4)	0.296	3 (4.3)	4 (5.3)	1.000
Total	244	197	0.269	175	122	0.051	69	75	0.687
Brazil									
CC				30 (45.5)	44 (55.7)	0.246			
CG				35 (53.0)	29 (36.7)	0.065			
GG				1 (1.5)	6 (7.6)	0.127			
Total				66	79	0.059			
Combined sample[a]									
CC	196 (51.6)	194 (56.1)	0.234	146 (50.9)	141 (57.1)	0.164	50 (53.8)	53 (53.5)	0.642
CG	172 (45.3)	126 (36.4)	0.016	133 (46.3)	85 (34.4)	0.006	39 (41.9)	41 (41.4)	0.754
GG	12 (3.2)	26 (7.5)	0.012	8 (2.8)	21 (8.5)	0.004	4 (4.3)	5 (5.1)	1.000
Total	380	346	0.005	287	247	0.001	93	99	0.970

[a]Combined sample also includes transmitted and non-transmitted genotypes from Azores.
[b]*P*-value was assessed using Fisher's exact test with the exception of the global *P*-value that was obtained by χ^2 test with 2df. Fisher's exact test were derived from a series of simple 2×2 tables based on the frequency of each genotype versus all others combined between the case and control groups. *P*-values$<$0.05 are shown in bold.

geno-PDT, which allows the detection of interaction between alleles at a single locus, we found a significant under-transmission (*P*-value=0.034) of the genotype GG at the marker rs7113041 (Table 6.3). After stratifying probands by gender, the transmission deficit of the GG genotype was maintained for males (*P*-value=0.014) but not for females (Table 6.3).

In order to increase the power of the rs7113041 analysis, we pooled the cases from the three different samples and pooled the controls from Portugal-mainland and Brazil with the genotypes made of non-transmitted alleles from the Azorean triads (Table 6.2). Such pooling can be justified because all three independent samples have a common European origin and because no difference was found when genotype frequencies of cases or controls were compared between the three samples. In this combined analysis, the genotype distribution showed significant differences between schizophrenic patients and controls in males, but again this difference was not detected in females. Specifically, the CG association found in males from Portuguese-mainland and the GG association found in the Azorean male offspring were both present in the

Table 6.3: Transmission of marker rs7113041 in Azorean triads with schizophrenia.

Mating	Total sample (*n*=70)			Male Offspring (*n*=46)			Female Offspring (*n*=24)		
Type	CC	CG	GG	CC	CG	GG	CC	CG	GG
CC×CC	16	-	-	10	-	-	6	-	-
CC×CG	14	20	-	9	11	-	5	9	-
CC×GG	-	6	-	-	4	-	-	2	-
CG×CG	5	6	1	5	6	0	0	0	1
CG×GG	-	2	0	-	1	0	-	1	0
GG×GG	-	-	0	-	-	0	-	-	0
n (%)	35 (50.0)	34 (48.6)	1 (1.4)	24 (52.2)	22 (47.8)	0 (0.0)	11 (45.8)	12 (50.0)	1 (4.2)
TDT	35 T/37 NT	34 T/26 NT	1 T/7 NT	24 T/21 NT	22 T/19 NT	0 T/6 NT	11 T/16 NT	12 T/7 NT	1 T/1 NT
P-value[a]	0.752	0.182	0.034	0.549	0.513	0.014	0.197	0.197	1.000

[a] *P*-values were obtained by Geno-PDT program; T=Transmitted; NT=Non-transmitted.

combined male sample (Table 6.2). In this combined sample, the CG genotype was found to be over-represented in male patients (*P*-value=0.006; OR=1.65; CI=1.16 to 2.34), while the GG genotype was more frequent in male controls (*P*-value=0.004; OR=0.31; CI=0.13 to 0.71). In the same way when the effect of CC+CG together was analyzed against GG, a predisposition to develop schizophrenia was observed (*P*-value=0.004; OR=3.24; CI=1.41 to 7.46). Allelic distribution remained not associated.

The haplotype analysis of the two markers in linkage disequilibrium (i.e., rs7113041-rs12541) did not show genetic association with schizophrenia when overall frequencies were compared in any of the three samples analysed. Although we found association of specific classes of geno-haplotypes and haplotypes after gender stratification, the obtained results were not more informative than the ones reported in the genotypic analysis (*P*-values$>$0.028). These additional results are available upon request.

Hardy-Weinberg equilibrium was determined for the three SNPs tested in the three samples analyzed, and no deviation was found in controls, parents or overall groups. A deviation was found in the male patients from Brazil and Azores, and a borderline *P*-value was found in males from Portugal-mainland, which could in fact be a result of the association we described above [407].

6.4 Discussion

To date, the chromosomal region in which the *NRGN* gene is located has been implicated in schizophrenia by a single genome-wide linkage study [132]. The

present study provides evidence implicating the *NRGN* gene itself in schizophrenia. Specifically, the rs7113041-CG genotype showed significant differences between male patients and male controls in the Portuguese-mainland sample, with this genotype predisposing to the disease. The Brazilian sample shows the same tendency. Since population admixture is a legitimate concern for positive findings in case-control association studies [79], we performed a family-based association study on an independent sample from the Azorean Islands, and a deficit of transmission of the rs7113041-GG genotype from parents to the affected male offspring was detected. In accordance with the results of the case-control analysis, no transmission distortion was detected to female offspring. Interestingly, the GG genotype seems protective against schizophrenia in the Azorean sample. The same tendency is observed in both case-control samples, even though not reaching statistical significance. This might be related to the sample size since in the combined analysis (all samples included) we did detect a significant association of the GG genotype.

We tested 3 SNPs located in the *NRGN* gene in two case-control samples plus one parents-offspring sample. This procedure amounts to multiple testing, and formally *P*-value correction needs to be applied. However, the appropriate method of correction for multiple comparisons in candidate gene association studies remains unclear [343]. Nevertheless, the fact that we find a nominally significant (at P-value<0.05) association of rs7113041 CG and GG genotypes in two independently ascertained populations (males from the Portuguese-mainland and in the Azorean male-offspring), and highly significant (P-value=0.001) association in the pooled male sample, gives us confidence in our finding.

Particularly intriguing is the fact that we found a gender related association in both family-based and case-control samples, which may be related to phenotypic sex differences observed in schizophrenia. It is known that schizophrenia presents a gender bias in age of onset, severity, clinical course, and response to antipsychotic drugs [10, 208], and that some neuroanatomical changes in schizophrenia are sex-dependent [149]. However, to our knowledge, NRGN has never been reported to mediate such phenotypic differences. Larger samples and stratification by several disease features will be needed

to establish whether the association is in fact due to gender or to some specific symptom mediated by NRGN that is more frequent in males. The gender bias we found is not unprecedented, since other schizophrenia studies also identified gender-specific effects on genes such as DISC1, ZDHHC8, and COMT [145, 273, 342].

The implicated SNP rs7113041 is located in a noncoding region, but because it is in the vicinity of the TRE it can interfere with the binding of the thyroid hormone receptor and subsequently influence the transcription of the *NRGN* gene. Functional assays will be needed to analyze the impact of the rs7113041 polymorphism on the NRGN expression. Of relevance is the recent finding of decreased NRGN protein levels in the prefrontal cortex of schizophrenic patients [40]. Since 6 out of the 7 brains used in that study are from males, it will be of interest to verify whether the rs7113041 genotype of those individuals relates with the altered protein levels reported.

NRGN is a new player in the glutamatergic hypothesis of schizophrenia. Several initial clinical observations support that the pathophysiology of schizophrenia involves an NMDA receptor hypofunction [368]. NMDA receptor antagonists, such as phencyclidine (PCP), ketamine, and MK-801, mimic schizophrenia in healthy individuals [191] or exacerbate symptoms in schizophrenic patients [193]. Moreover, treatment with the atypical antipsychotic clozapine attenuates exacerbation of clinical symptoms produced by ketamine [222]. Similarly, schizophrenia-like behavior in mice with reduced levels of NMDA receptor expression (5% of the normal level) can be reverted in part by the administration of antipsychotic drugs [259]. In addition, agents that act as agonists at the glycine modulator site on the NMDA receptors improve negative symptoms and have a variable effect on cognitive and positive symptoms in schizophrenic patients [369]. Interestingly, kynurenic acid, an endogenous antagonist of NMDA receptor, has been reported to be increased in the hippocampus of schizophrenics in postmortem studies [334].

Analysis of the glutamatergic transmission places NRGN in the center of the glutamatergic pathway. Figure 6.2 summarizes the relationship of NRGN with the glutamatergic hypothesis of schizophrenia. On one hand, NRGN is a downstream target of glutamatergic transmission. Thus the hypofunction of

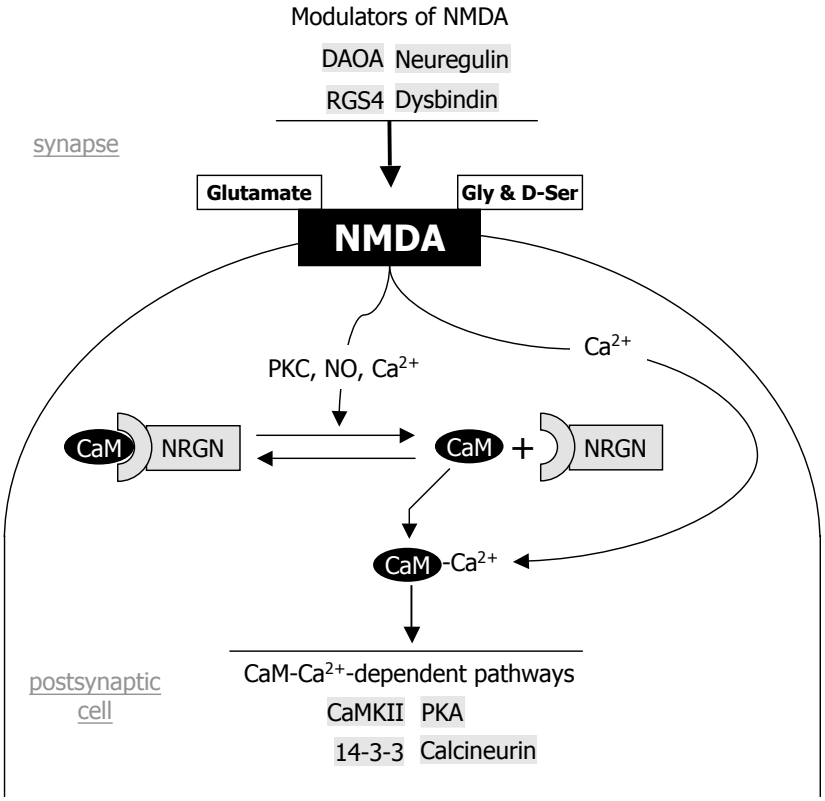

Figure 6.2: Summary of the relationship of NRGN with the glutamatergic hypothesis of schizophrenia

the NMDA receptor present in schizophrenia will impair NRGN phosphorylation/oxidation. On the other hand, by regulation of intracellular levels of Ca^{2+} and CaM-Ca^{2+}, disruption of NRGN homeostasis will alter postsynaptic signal transduction pathways, which are regulated by these secondary messengers. Interestingly, schizophrenic postmortem brains have altered expression levels of CaM-Ca^{2+} target genes. While PKC [71], calcineurin [87] and 14-3-3 [249] were found decreased in the temporal lobe, hippocampus, and prefrontal cortex, respectively, CaMKII levels were found increased in the prefrontal cortex of schizophrenic brains [281]. This can be a consequence not only of the NMDA receptor hypofunction but also of the reduced NRGN levels [40]. Altered NRGN activity could ultimately result in the dendritic and spine loss described in schizophrenia [39, 111, 118] as suggested by Broadbelt and collaborators [40].

An interesting feature in the relation between glutamatergic neurotransmission and schizophrenia, that can be explained at least in part by NRGN function, is the observation that the psychotomimetic effects of NMDA antagonists are minimal or absent in children but become apparent in late adolescence and early adulthood [402], the time when schizophrenia symptoms typically have their onset. This could reflect a direct influence of hormonal status in the NMDA receptor or in any of its downstream effectors, like NRGN which is supported by the age-related down-regulation of NRGN expression observed in rodents [99, 266]. In addition, several modulators of NMDA receptor activity, implicated in schizophrenia, could exert their effect, at least in part, by altering NRGN activity. Among these are dysbindin, neuregulin, D-amino acid oxidase activator (DAOA), and regulator of G-protein signaling 4 (RGS4), all strong candidates in the pathophysiology of schizophrenia [142].

In conclusion, we have found an association of the *NRGN* gene with schizophrenia in males. Given NRGN's known functions, one may speculate that it plays a role specifically in the cognitive deficits observed in the disease. Future work needs to clarify the significance of this association.

7

Thyroid hormone function in schizophrenia - a preliminary study

Abstract

Several lines of evidence support that schizophrenia arises from the cumulative effect of many risk factors that in combination disrupt the normal brain development. Both genetic and environmental factors are believed to interplay in the etiology of schizophrenia.

Given the importance of thyroid hormones in the normal development of the central nervous system, it has been suggested that abnormal levels of this hormone, during critical periods of brain development, could explain the deviations from normal neurodevelopmental found in schizophrenic patients.

Even though the thyroid hormone levels in adults will not necessary reflect the values present during embryonic development they may provide an indication. In the present study we investigate the serum levels of thyroid hormones in schizophrenic male patients and compare them with mentally healthy individuals. The levels of triiodothyronine (T_3) in its free form were found significantly decreased in schizophrenic male patients. We also observed higher prevalence of thyroid disorders among mothers of patients. Together, these two preliminary observations suggest that patients may have been exposed to TH deficiency during gestation, which, if confirmed, reinforces the neurodevelopmental hypothesis of schizophrenia.

When comparing stable patients with patients in crisis, the levels of total T_3 were found decreased in the latter. Since both groups are under medication, this decrease may be associated to manifestation of a particular symptom, which should be further investigated.

7.1 Introduction

Schizophrenia is a disabling mental disorder that affects about 1% of the population worldwide [171]. Schizophrenic patients present brain abnormalities that are likely to have a neurodevelopmental origin [225]. Epidemiological studies clearly indicate that genetic factors play an important role in the etiology of

schizophrenia [239], and that these might interplay with environmental risk factors to lead to disease onset [68, 42, 358, 154].

Recent findings suggest that prenatal nutritional deprivation may be an environmental risk factor for schizophrenia [350, 358], although it is not known whether the predisposition increases due to general malnutrition or lack of a specific micronutrient. Among the essential micronutrients is iodine, whose dietary intake is required by the thyroid gland for thyroid hormone (TH) production (for review see [86]). THs are necessary for the proper development of the central nervous system (CNS), with an increased demand of iodine during pregnancy [58, 120, 268, 72]. Severe hypothyroidism of the mother during pregnancy may result in spontaneous abortion, stillbirth, congenital abnormalities, and some forms of mental retardation, such as cretinism [85, 268]. The severity and irreversibility of CNS damage is dependent on the degree of TH abnormality, the time during development at which it occurs, and its duration.

The impact of mild or even subclinical hypothyroidism during pregnancy on adult human behavior is still under discussion and was hypothesized to participate as a predisposing factor in schizophrenia [293]. Because TH levels are dependent, both on dietary supply of iodine and on genetically determined enzymes and other proteins that regulate TH metabolism, abnormal levels of THs during critical periods of brain development could constitute a link between genetic and environmental susceptibility factors. In other words, schizophrenia may be associated with maternal thyroid deficiency at specific critical times during gestation.

In addition to its involvement in the developing brain, significant disturbance of thyroid function in the mature brain is known to profoundly alter mental processes, including cognition and emotion. In the adult life, both hyperthyroidism and hypothyroidism in severe forms have been associated with clinical symptoms of psychosis that frequently resemble schizophrenia [404, 403]. In particular, it has been shown that about 10% of hospitalized patients with acute psychosis have some abnormality in thyroid function [405, 321].

In fact, several studies in schizophrenic patients have described altered TH serum levels [315, 26, 229, 288, 307, 344]. Some reports have noted that TH levels are increased only in the acute unmedicated phase of psychotic illness

and decrease with the response to medication [26, 315, 415].

In order to further study the involvement of TH in schizophrenia, the present study evaluates the thyroid function in chronic male schizophrenia patients and compares them with mentally healthy individuals. To our knowledge, no such study has so far been performed in the Portuguese population.

In particular, we measured serum levels of thyroid-stimulating hormone (TSH), total and free thyroxine (TT_4 and FT_4), and total and free to triiodothyronine (TT_3 and FT_3). TSH, produced in the pituitary gland, stimulates virtually all steps of TH production. T_4 is the main product of the thyroid gland. Although a small percentage of T_4 is converted to T_3 within the thyroid gland most T_3 in circulation is derived from extrathyroidal conversion of T_4 in the peripheral tissue, mostly in the liver and kidney [188]. Once released into circulation, T_4 and T_3 are rapidly bound to plasma proteins [314]. Less than 0.5% of the total TH in circulation is in its free form [62].

7.2 Material and methods

Twenty one schizophrenic patients and 25 mentally healthy subjects were included in this study. Ages are matched between cases (32.8 ± 10.0) and controls (29.7 ± 6.8). All participants in the study were males with a European ancestry. None of the participants had a known history of thyroid dysfunction, but 8 of the patients (38%) reported a close relative (their mother in 4 cases) with history of thyroid hormone disorders, while this was the case for only one of the controls (4%).

All schizophrenic subjects were patients from the hospitals of Coimbra and Braga, Portugal, and the study was approved by the respective ethic committees. All were receiving medication and seven (33.3%) were in crisis at the time of the study, while 10 (47.6%) were in a stable phase. The average age of onset was 19.1 ± 5.4, ranging between 13 and 27 years old. The average duration of the illness was 13.6 ± 8.6 which ranges from 1 to 36 year.

All subjects in the study were evaluated using the Diagnostic Interview for Genetic Studies (DIGS) [283], a semi-structured interview that assesses the

criteria for schizophrenia and other psychiatric diseases. All patients where then diagnosed as having schizophrenia according to Diagnostic and Statistical Manual of Mental Disorders, review of the third edition (DSM-IIIR) criteria [11].

After obtaining informed consent, blood was drawn from subjects, and the serum was separated and frozen until hormone measurement. For assessing thyroid hormone function serum measurements of TSH, TT_4, FT_4, TT_3, and FT_3 were performed, using radioimmunoassay in accordance with the procedure described in the manufacturer's instructions (Diagnostic Products Corporation, LA, USA).

Student's t-test was used to compare serum levels and age distributions between patients and controls. In addition, this test was used to compare serum levels between patient groups, when subdividing according to response to neuroleptic medication, stable/crisis status at the time of study, and family history of thyroid disease. The relationship between variables was analysed using Pearson's correlation coefficient. All tests were two-tailed and results were considered to be significant if the *P*-value was less than 5%.

7.3 Results

All concentration levels measured fell within the considered normal range for TH function. As shown in Table 7.1, the serum concentrations of TT_4, FT_4, TT_3, and TSH measured for patients were not significantly different from those in the control group. In contrast, the FT_3 serum levels were significantly lower in schizophrenic patients.

When comparing TH serum levels between patients and their state of disease at the time of the study, the levels of TT_3 were found to be significantly (*P*-value=0.21) decreased in patients that were in crisis (107,3$\pm$14,2) with respect to those that were stable (133,1$\pm$26,4).

Among schizophrenic subjects, no relationship was detected between serum TH concentrations, age of onset, duration of illness, family history of thyroid disease, or response to neuroleptic medication.

Table 7.1: Age, serum concentration of TT_4, FT_4, TT_3, FT_3, and TSH.

		N	Mean±SD	*P*-value
Total T_4 (mg/dL)	Cases	20	7.3±1.1	0.263
	Controls	25	7.6±0.9	
Free T_4 (ng/dL)	Cases	17	1.5±0.2	0.158
	Controls	25	1.6±0.2	
Total T_3 (ng/dL)	Cases	21	121±23	0.736
	Controls	25	123±13	
Free T_3 (pg/dL)	Cases	17	3.3±0.5	0.044
	Controls	25	3.7±0.5	
TSH (mIU/mL)	Cases	20	1.5±0.8	0.211
	Controls	25	1.3±0.4	

7.4 Discussion

Though still within the normal range, the serum levels of FT_3 were found to be significantly decreased in our patients. Note that for most hormones “normality´´ is defined as a broad range of values, and several studies suggest that significant difference between certain clinical groups may in fact reflect some symptom or clinical feature of the disease. The control mechanisms by which the hypothalamus-pituitary-thyroid axis regulates the production of TH by the thyroid gland, are subtle and complex. Therefore, small fluctuations such as those we found may still reflect or contribute to the schizophrenia manifestation.

The fact that in particular FT_3 was found to be altered is interesting, since THs exert their physiological effects mainly by binding of T_3 to specific nuclear receptors, which are ligand-dependent transcription factors. Furthermore, the alteration of FT_3 levels in particular is noteworthy because the concentration of the free forms, rather than the total TH, is considered a more accurate indicator of thyroid hormone-dependent activity in cells [314].

It is unknown how such subtle decrease of FT_3 could impact TH function, in particular the regulation of gene expression by T_3, and lead to psychiatric disorders such as schizophrenia. However among the genes whose expression is regulated by T_3 are several that have been found decreased in schizo-

phrenic brains, such as those encoding for myelin basic protein [317], and for neurogranin [40] (discussed in Chapter 6).

Since most T_3 is derived from deiodination of T_4 within target cells [188], a decrease in the T_3 circulating fraction may reflect impaired deiodination. However, this might not be the case since no differences were found in serum TT_3 levels. It could instead result from altered tissue uptake or from abnormal binding to serum carrier proteins.

Other studies also report euthyroid abnormalities in schizophrenia [315, 26, 229, 288, 307, 344]. Unfortunately, disease classification and treatment differs between the studies and comparisons are therefore difficult to make. Interestingly, some of these studies report that acute, unmedicated patients have increased levels of TT_4 and FT_4 that decrease upon antipsychotic treatment [26, 315, 415].

It is known that antipsychotic drugs may influence TH levels [95]. Dopamine and THs have many synergistic effects in many metabolic processes [404]. Dopamine administration has been shown to rapidly decrease TSH secretion [329, 176], and conventional antipsychotics, which are known to act mainly by blocking various types of dopamine receptors, can inhibit TSH secretion [415].

Serum levels of TT_3 were found decreased for those patients that were in crisis at the time of study. All patients in our sample are under chronic treatment and, therefore, the observed TT_3 decrease can not be attributed to treatment, but may rather result from a particular feature of the disease. To interpret this finding, the interaction between dopamine and TH might be relevant. The CSF levels of dopamine metabolites has been shown to correlate negatively, not only with TSH, but also with TT_3 [354]. Thus, the observed TT_3 decrease during crisis can be explained by increased dopamine transmission, since such increase is known to induce schizophrenia symptoms.

The increased prevalence of thyroid disorders in relatives of schizophrenic patients, as observed in our sample, has already been reported by others [74], which further supports a possible link with the disease. Note that 4 of the 8 patients´ relatives with thyroid disorder in our sample, are their mothers. Interestingly, recent studies have revealed that offspring of mothers who had TH de-

ficiency during pregnancy, even if subclinical, showed significantly lower performance in cognitive tasks when compared with matched controls [133, 179]. Intellectual dysfunctions have been documented to occur in schizophrenia patients even prior to the onset of psychosis [69].

An interesting extension of the preliminary study we reported here is to include the mothers of the subjects into the study, and measure their thyroid function. In this way, not only diagnosed thyroid disorders, but also mild and subclinical hypothyroidism can be assessed. Such extended studies should be conducted on samples of larger size, allowing subdivision for instance by type of medication.

As discussed in the introduction, schizophrenia may be associated with maternal thyroid deficiency during specific critical times in gestation. Presently, our lab is conducting a study in pregnant women in whom TH function is evaluated and related to the newborn's psychodevelopmental performance. Follow-up of these children might provide further insights into whether altered TH function during pregnancy increases the risk for schizophrenia.

8

General discussion and future perspectives

In Chapter 1, we described the schizophrenia disorder and presented the thyroid hormone (TH) and retinoid hypotheses, which fit in the commonly accepted view that schizophrenia is a neurodevelopmental disorder. These hypotheses say that TH and retinoid abnormalities during critical periods of neurodevelopment could lead to brain alterations that give rise to schizophrenia later in life.

In Chapters 3 to 6 we reported on association studies of four candidate genes against the background of the TH and retinoid hypotheses. Our strategy of investigation consisted of selecting candidate genes based on their participation in the TH or retinoids pathways, and their involvement in functions known to be abnormal in schizophrenia. In the remainder of this chapter, we will give the rationale for the selection of each gene, and we will summarize and discuss the relevance of the various results obtained (Sections 8.1 to 8.4). Subsequently, we will propose a hypothetical mechanism of action of how a *neurogranin* variant could result in increased susceptibility to schizophrenia (Section 8.5). We discuss how the results of the preliminary study described in Chapter 7 could corroborate this mechanism. In Section 8.6, we suggestion possible lines of future work.

8.1 Nur-related receptor 1 (NR4A2)

NR4A2 is an atypical member of the nuclear receptor superfamily that includes mostly ligand-activated receptors, like the retinoid and TH receptors, which regulate gene expression via recognition of specific DNA-binding sequences [119, 200]. Unlike other nuclear receptors, NR4A2 does not seem to need any ligand to activate the transcription of target genes [322], for which reason it is called an orphan receptor. Retinoid X receptor (RXR) has been shown to interact directly with NR4A2, by forming heterodimers, and to modulate its transcriptional activity [322, 295, 1].

The *NR4A2* gene has been related with dopamine transmission known to be abnormal in schizophrenia [84]. NR4A2 is highly expressed in dopaminergic neurons of the midbrain [418, 328, 55]. Disruption of *Nurr1* (the mouse

homologue of *NR4A2*) revealed that its expression is essential for the formation of the dopaminergic system [418, 328], via regulation of dopaminergic neuron-specific genes such as *tyrosine hydroxylase* [326, 185] and the *dopamine transporter* [55, 24]. NR4A2 is also thought to protect dopamine neurons against toxic insults, thus promoting their survival [204]. Due to its important role in the modulation of the dopaminergic neurotransmission, NR4A2 may be implicated in disease states where dopaminergic neurotransmission is dysregulated, such as schizophrenia. In fact, the 2q22-23 chromosome region that harbors the *NR4A2* gene has been suggested to be implicated in schizophrenia by linkage studies [260, 408, 75].

Several studies have failed to detect association between the *NR4A2* gene and schizophrenia [52, 168, 170]. However, given the well-established role of NR4A2 in the formation of the dopaminergic system any mutation that alters the function of this gene is expected to significantly impact brain function, which can lead to mental illness such as schizophrenia.

Six rare variants have been described for the *NR4A2* gene [44, 205, 60], as shown in Figure 8.1. The mutations c.289A>G, c.308A>G and c.366-369delTAC, found in schizophrenic and manic depressive individuals, originate amino acid changes at the protein level and result in decreased transcriptional activity of NR4A2 dimers [44]. Decreased transcriptional activity of *NR4A2* is also described for two mutations (c.-291delT and c.-245T>G) in the 5´ untranslated region found in patients with Parkinson's disease [205]. The c.-469delG mutation, in the promoter region, was found in schizophrenic patients and its effect on gene transcription has not been determined [60]. Thus, these mutations may be expected to impair proper function of NR4A2, leading to impaired transcription activity in its downstream target genes, such as *tyrosine hydroxylase* and *dopamine transporter*.

We investigated the presence of these six variants in a Portuguese and in a Brazilian sample (Chapter 3). None of the variants were found in any of the schizophrenic patients or mentally healthy individuals.

The fact that the variants were not present in any of the 448 individuals tested confirms that these mutations are indeed very rare. This absence also implies that, in the Portuguese and Brazilian populations, none of the variants

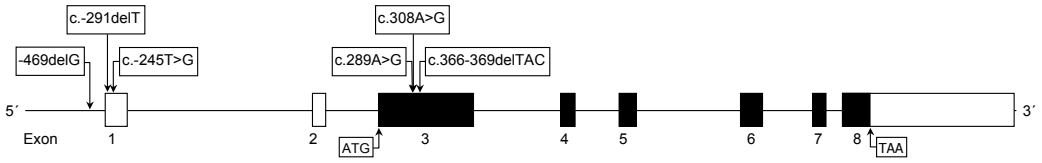

Figure 8.1: Genomic structure of the *NR4A2* gene and positions of the six DNA variants analyzed. Black boxes represent protein-coding regions and white boxes represent untranslated regions. Locations of initiation (ATG) and stop (TAA) codons are also provided.

plays a major role in the etiology of schizophrenia. However, our negative results do not exclude the possibility that mutations on the *NR4A2* gene could account for high genetic susceptibility to schizophrenia in the particular individuals where the mutations were detected in the original studies.

Since altered expression of genes could result not only from DNA variants but also from inadequate supply of modulators of their transcription activity [280], the fact that we failed to detect the presence of the studied DNA variants in our samples does not exclude the possibility that the *NR4A2* expression levels are altered in the brains of patients. In fact, data indicating decreased expression in schizophrenic patients has been submitted to the public genomic database of the Stanley Medical Research Institute [148]. Below we will comment more elaborately on possible future work exploiting this database information (Section 8.6).

8.2 Lipocalin-type of prostaglandin D2 synthase (PTGDS)

The PTGDS protein is abundantly present in the cerebrospinal fluid (CSF) [412]. The relation of PTGDS with THs and retinoids is reciprocal. On the one hand, both regulate the expression of the *PTGDS* gene [175, 110, 109]. On the other hand, since PTGDS has been described as a transporter for THs and retinoids [362, 31], abnormal levels of PTGDS can eventually disturb their availability for modulating the expression of a large number of genes, including PTGDS itself. In this way, PTGDS could contribute to the deleterious effects of altered THs and retinoids in the central nervous system.

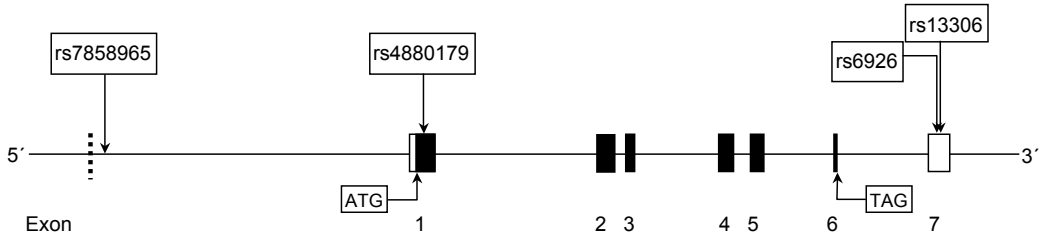

Figure 8.2: Genomic structure of the human *PTGDS* gene and location of the four SNPs detected. Black boxes represent protein-coding regions, white boxes represent untranslated regions, and the dashed line represents the TH response element (TRE). Locations of initiation (ATG) and stop (TAG) codons are also shown.

Several lines of evidence support that schizophrenic patients have altered lipid metabolism [101], including decreased levels of arachidonic acid [414]. Arachidonic acid and its products (e.g. prostaglandins) are critical to various signal transduction pathways [35]. The abnormal niacin response found in schizophrenic patients [159, 393, 345] might be due to decreased levels of arachidonic acid [101] or can result from abnormal activity of the enzymes responsible for the synthesis of PGD2, whose epidermic release is involved in the flushing response to niacin [271, 270].

The previous suggests the existence of abnormal prostaglandin signaling in schizophrenia patients. In the brain, the formation of PGD2 is dependent on PTGDS [375] and, interestingly, decreased levels of PTGDS have been described in the CSF of schizophrenic patients [139, 137, 138].

For these reasons, we studied the association of *PTGDS* to schizophrenia (Chapter 4). We first screened the exons, exon-intron boundaries, and the promoter of the gene to detect DNA variants. The four detected and previously described variants are shown in Figure 8.2.

Association studies were performed on three of the detected variants to determine whether they predispose individuals to develop schizophrenia, using two case control samples from the Portuguese mainland and from Brazil, and a parents-patient sample from the Azorean islands. The fourth variant (rs7858965) was not included in the study since it is in complete LD with one of the others.

None of the three variants was found associated to schizophrenia in any of

our three independent samples. Thus, our data do no support the involvement of the *PTGDS* gene in the schizophrenia etiology.

It remains to be verified whether the altered PTGDS levels detected in the CSF of patients are a consequence of disease manifestation or induced by antipsychotic medication. If these influences can be excluded, then the altered levels of PTGDS may be attributable to inadequate supply of THs and/or retinoids, which regulate the gene's expression. In this case, PTGDS could mediate the deleterious effect of TH and retinoids in the brain.

8.3 Transthyretin (TTR)

TTR is a major carrier of thyroxine (T_4) and it binds to retinol through association with retinol binding protein (RBP), both in the serum and in the CSF [292]. As a carrier of THs and retinoids, TTR indirectly mediates the transcription of several of their target genes and, in this way, TTR may contribute to the harmful effects of irregularities in their supply.

TTR has been implicated in behavior by observations that *TTR*-null mice present increased motor activity in behavior tests addressing anxiety-like and depression-like behaviors [349]. Furthermore, decreased levels of TTR in the CSF were found in patients with depression [357] and with Alzheimer's disease [337].

These considerations prompted us to study the association of *TTR* with schizophrenia (Chapter 5). We screened the regulatory and coding regions of the gene to detect DNA variants. The two variants detected, one of which we described for the first time, are shown in Figure 8.3.

We studied possible association of the two variants in our two case control samples from the Portuguese mainland and from Brazil, and a parents-patient sample from the Azorean islands. We also analyzed the circulating levels of TTR itself and of RBP.

Neither variant was found associated with schizophrenia in any of the three independent samples. Thus, our data do not support the involvement of the studied *TTR* variants in the schizophrenia etiology.

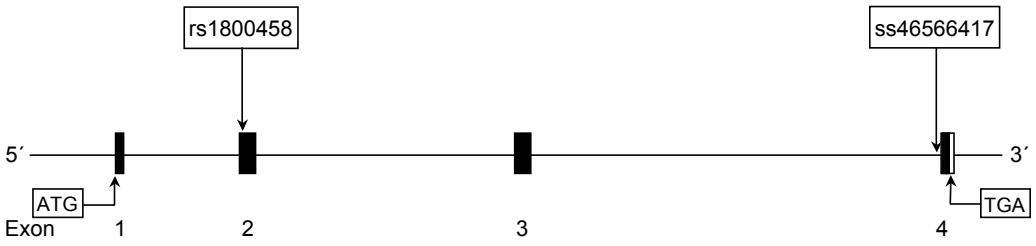

Figure 8.3: Genomic structure of human *TTR* gene and locations of the two SNPs analyzed. Black boxes represent protein-coding regions, white boxes represent untranslated regions. Locations of initiation (ATG) and stop (TGA) codons are also provided.

Likewise, the measurements of serum levels of TTR and RBP did not reveal any alteration associated to the disease.

Our results may seem to be in contrast with another study in which the serum levels of RBP were found decreased in the serum of Chinese schizophrenic patients from Singapore [409]. However, in that study, the RBP decrease was accompanied by a decrease of albumin. Since RBP is a useful marker of protein malnutrition and both are inflammatory acute phase proteins, the values observed in that study might reflect a particular nutritional or inflammatory state in the patients, rather than schizophrenia manifestation.

It should be noted that serum levels of TTR do not necessarily reflect CSF levels since serum TTR originates primarily from the liver, whereas CSF TTR is mainly produced by and secreted from the choroid plexus [9]. In an early study, CSF levels of TTR were found to be unaltered in schizophrenia patients [34].

Interestingly, after our study three further studies reported decreased levels of TTR both in the blood [413, 155] and in the CSF [155, 388] of schizophrenic patients. This discrepancy with the earlier negative results of CSF measurements [34] could stem from the fact that the control group in this earlier study consisted of patients with minor psychiatric problems, rather than mentally healthy individuals.

The discrepancy of these three recent studies with the lack of alteration found by us may stem from differences in medication. Patients medicated with chlorpromazine were reported to have increased TTR serum levels compared with drug-naïve patients [388], while medication with clozapine was described not to alter TTR plasma levels in patients [413]. Naïve patients were consis-

tently found to have decreased levels of TTR in CSF and serum [155]. Furthermore, this last study reports decreased levels of TTR in prefrontal cortex of medicated patients. The fact that we did not find decreased serum levels of TTR may be attributable to the fact that our patients are under medication. However, because medication programs are mixed, and different between patients, our data did not allow to appropriately differentiate groups of patients. Future studies are needed to confirm the influence of medication in TTR expression. Evaluation of the levels of gene transcription in mice submitted to various kinds of neuroleptic drugs could shed light on the matter. In particular, the effects of clozapine, reported not to affect TTR serum levels in schizophrenic patients [413] but to induce TTR expression in the rat brain [59], should be clarified.

One of the *TTR* polymorphisms we studied, ss46566417, had not been reported before. This novel polymorphism is located on intron 3, 18bp upstream of exon 4 of the gene. Since it is located near to a splicing site it may interfere with transcription. For 100 individuals from our Portuguese and Azorean samples, both genotype data and TTR serum level measurements were available. These individuals include cases and controls of both genders. We found that the G allele was present in heterozygous form in 7 of these individuals and that the level of TTR in this group was increased (331 ± 50 versus 287 ± 45 mg/L), with a borderline significance (*P*-value=0.059). The homozygous form was not present. Due to the low frequency of the G allele, a larger sample size is needed to confirm this tendency. The larger sample should also allow stratification by gender and by phenotype. Thus, a future study on a larger scale could confirm whether the G allele of this novel SNP indeed increases expression of *TTR*.

8.4 Neurogranin (NRGN)

The *neurogranin* (*NRGN*) gene encodes a postsynaptic brain-specific protein and its expression is regulated by THs and retinoids [93, 130, 161, 165].

NRGN binds calmodulin (CaM) with high affinity [108, 300, 303]. By regu-

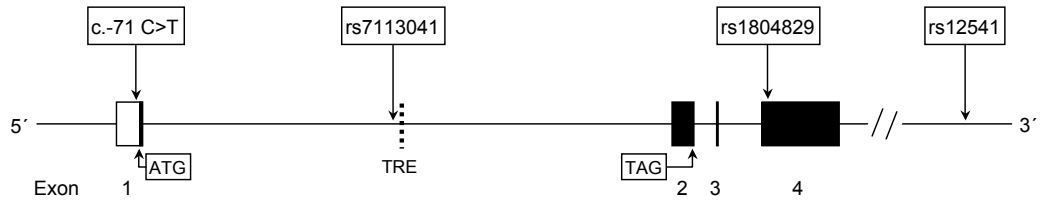

Figure 8.4: Genomic structure of the human *NRGN* gene and positions of the three SNPs tested and of the mutation found. Black boxes represent protein-coding regions, white boxes represent untranslated regions, and the dashed line represents the TH response element (TRE). Locations of initiation (ATG) and stop (TAG) codons are also provided.

lating the release of CaM, NRGN indirectly regulates the expression of CaM-Ca^{2+}-dependent enzymes that play an important role in the neuroplasticity mechanisms of learning and memory [157, 291]. The stimulation of N-methyl-D-aspartate (NMDA) receptors by glutamate induces dissociation of the NRGN-CaM complex. Therefore, altered NRGN activity could mediate the effects of NMDA receptor hypofunction suggested by several studies to be implicated in schizophrenia [368]. Moreover, the *NRGN* chromosome region has previously be implicated in schizophrenia by linkage studies [132].

These considerations prompted us to investigate the involvement of the *NRGN* gene in the schizophrenia etiology. We screened the exons and adjacent splicing sites, as well as the TH response element (TRE) on intron 1 of the *NRGN* gene to detect DNA variants. We detected a new single-nucleotide variant (c.-71C>T) and two previously described SNPs. These variants are shown in Figure 8.4, together with a third SNP obtained from the NCBI SNP database.

We studied association of the three SNPs of *NRGN* with schizophrenia in our three samples (Chapter 6). Because of its very low frequency, the c.-71C>T variant was not included in the association study. For two of the three variants studied (rs1804829 and rs12541), no association to schizophrenia was found in any of the three samples.

For males of Portuguese origin, genotype distribution showed association of the rs7113041 polymorphism to schizophrenia, and this result was confirmed in the Azorean patient-parents triads. More specifically, we found a significant excess of the CG genotype in Portuguese males (P-value=0.029,

n=297), with this genotype predisposing for the disease. For males in the smaller Brazilian sample, the same tendency was observed but without reaching statistical significance (*P*-value=0.065 with n=145). In the Azorean patient-parents sample, under-transmission was found for the GG genotype, both in the overall sample (*P*-value=0.039, n=70) and in the male-patient subsample (*P*-value=0.014, n=46), but not in the female-patient subsample. Analysis of the three samples combined strongly confirmed genotype association of the rs7113041 polymorphism to schizophrenia in males.

Confirmation of our result in further independent samples, stratifiable by gender, is of prime importance.

The gender specificity of the association is of particular interest since significant symptomatic differences between sexes have been observed in schizophrenia. As mentioned in Section 1.1.1 schizophrenia presents a gender bias in age of onset, severity of clinical course, and response to antipsychotic drugs. Males show more negative symptoms and cognitive deficits [208]. These gender-specific phenotypic differences may reflect distinct genetic components in disease etiology. In fact, the gender bias found is not unprecedented, since other association studies in schizophrenia reported association of female gender with DNA variants of the *DISC1*, *ZDHHC8*, and *COMT* genes, and of male gender with another variant of *COMT* [342, 273, 145].

Given NRGN's involvement in cognitive functions [157, 291], the association we found of the rs7113041 SNP with schizophrenia in males could explain the observation of more cognitive deficits in male patients. To explore this hypothesis, larger samples, stratified by several disease features, will be needed to investigate whether the association found is due to some specific symptom.

Another line of future study is warranted to clarify the influence of the G allele of the rs7113041 variant on the expression of *NRGN* in brains of schizophrenic patients. The implicated SNP rs7113041 could influence the transcription of the *NRGN* gene by interfering with the binding of the TH receptor to its DNA response element. To investigate this possible influence on gene expression, postmortem studies are needed that correlate *NRGN* expression levels in patient brains with their rs7113041 genotype. Both genders should be included in the study to establish whether the altered gene expression, if found,

is sex-dependent.

The c.-71C>T variant of the *NRGN* gene, found in one heterozygous individual among 60 cases screened, had not been reported before. Due to its low frequency this mutation was not included in the association study, but it was genotyped separately in the samples from the Portuguese mainland and from Brazil. After genotyping, the mutation was identified, in heterozygous form, in 2 out of 313 cases (244 Portuguese mainland and 69 Brazilian) and was absent from the 295 controls (210 Portuguese mainland and 85 Brazilian).

The fact that the mutation was found only in patients suggests that in the families of these two individuals this mutation could play a role in the etiology of schizophrenia. Therefore, we directed our attention to the relatives of these two individuals, in which the mutation is likely to occur with higher frequency than in the general population. The fact that the healthy mother of one patient was found to carry the mutation, rules out that presence of this mutation is sufficient for schizophrenia development, but is inconclusive regarding a potential causative effect in the context of a multifactorial etiology of schizophrenia. Screening of additional relatives might be essential to clarify the implication of the mutation in the schizophrenia disorder, but, unfortunately, none have so far been localized.

NRGN and the glutamatergic hypothesis of schizophrenia

NRGN joins several other genes already implicated in schizophrenia that are likewise involved in the glutamatergic pathway, such as dysbindin and neuregulin. Thus, our finding introduces an additional player into the glutamatergic hypothesis of schizophrenia.

Analysis of the glutamatergic transmission places NRGN in the center of the glutamatergic pathway. Figure 6.2 in Chapter 6 summarizes the relationship of NRGN with the glutamatergic hypothesis of schizophrenia. On one hand, NRGN is a downstream target of glutamatergic transmission. Thus the hypofunction of the NMDA receptor present in schizophrenia will impair NRGN phosphorylation/oxidation. On the other hand, by regulatiing intracellular levels of Ca^{2+} and CaM-Ca^{2+}, disruption of NRGN homeostasis will alter postsy-

naptic signal transduction pathways, which are regulated by these secondary messengers.

Interestingly, schizophrenic postmortem brains have altered expression levels of CaM-Ca^{2+} target genes. While PKC [71], calcineurin [87] and 14-3-3 [249] were found decreased in the temporal lobe, hippocampus, and prefrontal cortex, respectively, CaMKII levels were found increased in the prefrontal cortex of schizophrenic brains [281]. This can be a consequence not only of the NMDA receptor hypofunction but also of the reduced NRGN levels [40]. Altered NRGN activity could ultimately result in the dendritic and spine loss described in schizophrenia [279, 10] as suggested by Broadbelt and collaborators [40].

An interesting feature in the relation between glutamatergic neurotransmission and schizophrenia that can be explained at least in part by NRGN function, is the observation that the psychotomimetic effects of NMDA antagonists are minimal or absent in children but become apparent in late adolescence and early adulthood [402], the time when schizophrenia symptoms typically have their onset. This could reflect a direct influence of hormonal status in the NMDA receptor or in any of its downstream effectors, like NRGN; which is supported by the age-related down-regulation of *NRGN* expression observed in rodents [266]. In addition, several modulators of NMDA receptor activity, implicated in schizophrenia, could exert their effect, at least in part, by altering NRGN activity. Among these are dysbindin, neuregulin, D-amino acid oxidase activator (DAOA), and regulator of G-protein signaling 4 (RGS4), all strong candidates in the pathophysiology of schizophrenia [142].

8.5 A hypothetical causative mechanism

As suggested by linkage studies, schizophrenia possibly results from the effect of several genes each conferring only a small increase in disease susceptibility. It is also plausible that their small contributions become important only under certain environmental conditions.

We make the case here that low levels of THs and retinoids during critical

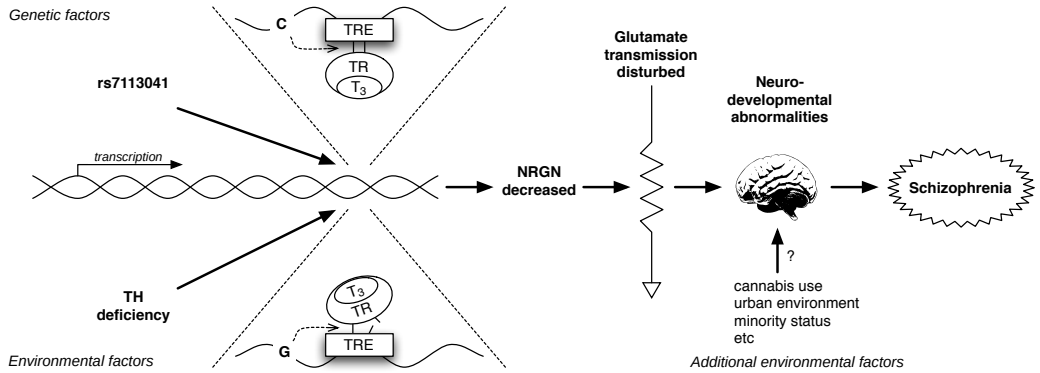

Figure 8.5: Hypothetical causative mechanism of how the rs7113041 polymorphism of *NRGN* may increase schizophrenia susceptibility. Under thyroid hormone (TH) deficiency, the common C allele of the rs7113041 polymorphism does not compensate the reduced transcription of the gene, provoked by the presence of the G allele, which interferes with the binding of the TH receptor to the TH response element (TRE). The decrease of NRGN levels result in disturbed glutamate transmission, leading to abnormal neurodevelopment, which may predispose to manifestation of schizophrenia later in life, possibly under the presence of additional environmental factors.

periods of brain development could interact with DNA variants in susceptibility genes to affect brain development negatively and give rise to schizophrenia later in life.

As we discussed before, the rs7113041 SNP of the *NRGN* gene could interfere with the binding of the TH receptor to the DNA response element and subsequently influence the transcription of the *NRGN* gene. Decreased expression of NRGN could seriously affect the glutamatergic transmission, leading to neurodevelopmental abnormalities which could consequently increase the risk to develop schizophrenia. The significant excess of the CG genotype in patients found in our study, with this genotype predisposing for the disease, could be explained by the possibility that the minor allele (G) of the rs7113041 SNP hampers binding of TR, thus inhibiting *NRGN* expression. This hypothetical mechanism is depicted in Figure 8.5. Note that individuals with the CG genotype who experience TH deficiency only when neurodevelopment has been completed may suffer abnormalities in CNS function through a similar decrease of NRGN transcription and the consequent disturbance of glutamate transmission.

The rs7113041 heterozygous genotype is also common in the general pop-

ulation. This is not surprising, if we assume that, in addition, TH levels are decreased during development of individuals who later develop schizophrenia. With normal levels of TH, the hampering effect of the G allele would be diluted by the presence of the normal C allele. But, when TH levels drop and general transcription slows down, the single C allele might fail to compensate, with subsequent decrease in *NRGN* expression.

Measurement of NRGN levels in postmortem brains, suggested above in order to investigate the influence of the G allele on gene expression, could shed light on this hypothesis. Note that, in case such study does not reveal altered expression, the rs7113041 variant may still be of influence on *NRGN* expression under decreased TH levels. To address this second possibility, *in vitro* studies should be conducted with cells in which reporter genes are introduced. By subjecting the cells to different levels of TH, their influence on gene expression in the presence of the C and G alleles of the rs7113041 polymorphism can be tested.

This hypothetical causative mechanism of how the rs7113041 polymorphism of *NRGN* confers susceptibility to schizophrenia can not explain that we found association to schizophrenia in males only, unless differences in TH regulation exist between the sexes. In Chapter 7, data of preliminary measurements of TH levels in circulation revealed decreased levels of free T_3 in male patients compared with controls. If these measurements in adults would reflect decreased levels during gestation, they would support our hypothesis. Additionally, this data, when combined with the *NRGN* genotyping data, reveal that free T_3 levels are decreased in male CG cases versus male CC cases, but that no such decrease is found for male controls. This observation likewise seems to support that the rs7113041 polymorphism could reduce expression of the *NRGN* expression under TH deficit, and thus increase schizophrenia vulnerability.

If in fact the G allele of the rs7113041 variant is associated with the decreased expression of *NRGN*, then the G homozygous will be selected against due to the low levels of *NRGN*. This would explain why the GG genotype is so rare. Only individuals with sufficiently high levels of THs would still be able to have minimally sufficient *NRGN* expression. This low frequency of the GG

genotype could also explain why, in our studies, we found this genotype to be protective rather than predisposing to schizophrenia.

In conclusion, under the hypothetical mechanism presented here, NRGN seems to provide an example of a gene-environment interaction with causative effects in the etiology of schizophrenia. Previously, the *APOE* and *HOPA* genes have been suggested as other examples of such gene-environment interaction.

APOE has been shown to increase susceptibility to schizophrenia in individuals subjected to fetal or early post-natal malnutrition [213], although it is not known whether the increased predisposition arises from a global nutritional deficiency or from a specific micronutrient deficiency. The latter seems to be the case, since *APOE* has also been shown to be a genetic risk factor for iodine deficiency disorders [218, 390] in regions with abnormally low supply of iodine [389]. Interestingly, the *APOE* gene has a T_4 binding domain, and it possibly mediates the binding of T_4 to low-density lipoproteins (LDL) [29]. In addition transcription of *APOE* has been shown to be upregulated by T_3 [378]. In conclusion, it seems valid to speculate that *APOE*, like *NRGN*, may confer increased risk to schizophrenia specifically under TH deficiency.

Recently the *HOPA* gene has been associated with an increased susceptibility to both schizophrenia and hypothyroidism [76, 297, 186, 296]. This association provides a possible genetic mechanism for the familial aggregation of thyroid function abnormalities and schizophrenia. The *HOPA* gene encodes a 230 kDa subunit of the TH-associated protein (TRAP) complex [169] which is responsible for tissue-specific transcription regulation [420, 258].

8.6 Future perspectives

In this section, we discuss ongoing and future lines of research.

8.6.1 Association studies

Association studies are a powerful tool to detect genes of small effect in disease susceptibility. With the objective of investigating whether retinoids and

THs are related to the pathophysiology of schizophrenia, we have performed association studies for four genes with various roles in the pathways and modes of action of THs and retinoids. For *NR4A2*, *TTR*, and *PTGDS*, we found no association to schizophrenia, but in the case of *NRGN*, association was found between the G allele of a particular variant with schizophrenia in males.

The decreased levels of free T_3 for which we detected a tendency in male patients indicates that further association studies are warranted for instance for genes encoding enzymes responsible for the regulation of T_3 levels. In particular, it seems promising to evaluate the association of *type II deiodinase* (*DIO2*) and *type III deiodinase* (*DIO3*), genes whose proteins regulate formation and degradation of T_3 in the brain (see also Section 1.2.4). Such studies are ongoing.

8.6.2 Expression studies

Association studies are an appropriate instrument to search for causal relationships between particular gene variants and disease manifestation. However, changes in gene expression underlying schizophrenia may result not only from variants in the DNA sequence of genes, but also from abnormal supply of modulators of the transcription activity of genes with unchanged DNA sequence [280]. This line of investigation was not included in the scope of the work reported in this thesis, but will be the subject of future studies.

The TH and retinoids hypotheses of schizophrenia involve exposure to abnormal levels of these elements during early stages of neurodevelopment. However, study of material harvested in early life, such as maternal serum and dried blood spots, meets with many practical obstacles, since it relies on banked specimens hard to obtain in sufficient quantity. Still, vestiges of alterations may remain in adult brain and provide clues on developmental impairments.

With the purpose of studying the expression of TH and retinoids genes in human brain material and lymphocytes, a microarray study has been designed and a corresponding platform is presently being constructed. The study in-

cludes genes that influence the level of THs and retinoids, genes than encode for TH and retinoid nuclear receptors, and genes whose expression is regulated by THs and retinoids.

The inclusion of lymphocytes in the study has several distinct advantages. Though blood can certainly not be regarded as a perfect reflection of the brain, recent evidence clearly shows that lymphocytes can at least partially represent or mimic brain dysregulation [117, 248]. Thus, lymphocytes can to some extent be used as an alternative to postmortem brain tissues, allowing for instance the screening of gene expression in drug naïve patients. The use of lymphocytes has the additional advantage that the material is easy to collect and any alteration found might eventually lead to a molecular diagnosis of the disease.

Recently, data from 12 microarray studies using brain tissues of patients with various mental illnesses, including schizophrenia, have been collected and made available in the online genomics database of the Stanley Medical Research Institute [148]. This database contains rich clinical information and gene expression patterns. In 11 of the 12 controlled studies, brains of schizophrenic patients were analyzed. The percentage of genes included in these 11 studies for which significantly altered expression was found amounted to 2.5%, considering an alteration to be significant if the *P*-value was less than 5%. This percentage drops to 0.8% when significance is accepted when the *P*-value is smaller than 1%.

We have retrieved information from this database for 176 of the genes included in the TH and retinoids microarray. For 49 out of the 176 genes, altered expression was detected, considering a *P*-value smaller than 5%. Out of those 49, a set of 8 genes was detected to have significantly altered expression even under *P*-value less than 1%. Thus, at these levels of significance, altered expression was detected in 27.8% and in 4.5%, respectively, of all 176 genes. These markedly higher percentages, with respect to the percentages for all genes found altered in schizophrenia, corroborate the hypothesis of abnormality of these metabolisms in schizophrenic patients.

Out of the 8 genes most likely to be altered, 5 are downregulated: *early growth response 1* (*EGR1*); *aldehyde dehydrogenase 1 family, member A2*

(*ALDH1A2*); *mitogen-activated protein kinase kinase 4* (*MAP2K4*); *nur-related receptor 1* (*NR4A2*); and *retinoic acid receptor, beta* (*RAR*β). The remaining 3 genes are upregulated: *lipoprotein lipase* (*LPL*); *RAR-related orphan receptor A* (*RORA*); and *tumor necrosis factor receptor superfamily, member 6* (*TNFRSF6*).

To confirm the alterations in the 49 genes, the microarray measurements need to be reproduced using a more sensitive and more accurate method of relative gene expression measurement, such as quantitative real-time polymerase chain reaction (RT-PCR). Such studies are currently under way for the set of 8 genes most likely to be altered. Apart from measuring gene expression, the levels of protein should also be measured because altered mRNA expression levels do not always lead to altered protein levels. This can be done using techniques such as immunohistochemistry in brain material.

For each gene for which altered expression is confirmed it must be clarified whether the alteration is the result of pharmacological intervention. This is especially important, since studies have shown that antipsychotic drugs seem to be capable of regulating TH and retinoid signaling pathways [196, 97]. Experiments with rodents submitted to different medication programs can help to address this issue. Gene expression measurements should be performed before and after administration of medication to conclude on the effect of each neuroleptic drug. These experiments are ongoing.

8.6.3 Epistasis

The basic strategy underlying most studies that aim to identify genetic risk factors for complex traits focus on individual *loci* which are assumed to contribute with small, additive effects to the phenotype. The studies reported in this thesis are no exception. However, given the limited number of genes in the human genome, it seems reasonable to assume that non-additive, or epistatic, interactions between genes contribute considerably to the inter-individual phenotypic variation [316]. Indeed, geneticists are increasingly aware of the possibility that gene-gene interactions are important determinants of complex traits [220, 224].

In fact, as pointed out in Chapter 1, the risk of developing schizophrenia, which decreases rapidly as the degree of genetic relatedness decreases, is compatible with a model of multiple *loci* with epistasic interaction between them. Since the various genes involved in the TH and retinoid metabolisms are biologically, functionally, temporally or spatially related, it is possible that they interact epistatically. This line of research merits future exploration.

Bibliography

[1] Aarnisalo P, Kim CH, Lee JW, Perlmann T (2002). Defining requirements for heterodimerization between the retinoid X receptor and the orphan nuclear receptor Nurr1. J Biol Chem 277(38):35118–35123.

[2] Abecasis GR, Cookson WO (2000). GOLD–graphical overview of linkage disequilibrium. Bioinformatics 16(2):182–183.

[3] Abecasis GR, Noguchi E, Heinzmann A, Traherne JA, Bhattacharyya S, Leaves NI, Anderson GG, et al (2001). Extent and distribution of linkage disequilibrium in three genomic regions. Am J Hum Genet 68(1):191–197.

[4] Abi-Dargham A, Gil R, Krystal J, Baldwin RM, Seibyl JP, Bowers M, van Dyck CH, et al (1998). Increased striatal dopamine transmission in schizophrenia: confirmation in a second cohort. Am J Psychiatry 155(6):761–767.

[5] Abu-Abed S, Dolle P, Metzger D, Beckett B, Chambon P, Petkovich M (2001). The retinoic acid-metabolizing enzyme, CYP26A1, is essential for normal hindbrain patterning, vertebral identity, and development of posterior structures. Genes Dev 15(2):226–240.

[6] Adler LE, Hoffer LJ, Griffith J, Waldo MC, Freedman R (1992). Normalization by nicotine of deficient auditory sensory gating in the relatives of schizophrenics. Biol Psychiatry 32(7):607–616.

[7] Aghajanian GK, Marek GJ (2000). Serotonin model of schizophrenia: emerging role of glutamate mechanisms. Brain Res Brain Res Rev 31(2-3):302–312.

[8] Albert M, Naeser MA, Levine HL, Garvey AJ (1984). Ventricular size in patients with presenile dementia of the Alzheimer's type. Arch Neurol 41(12):1258–1263.

[9] Aldred AR, Brack CM, Schreiber G (1995). The cerebral expression of plasma protein genes in different species. Comp Biochem Physiol B Biochem Mol Biol 111(1):1–15.

[10] Aleman A, Kahn RS, Selten JP (2003). Sex differences in the risk of schizophrenia: evidence from meta-analysis. Arch Gen Psychiatry 60(6):565–571.

[11] American Psychiatric Association (1987). Diagnostic and Statistical Manual of Mental Disorders (DSM-III-R) (3rd revised ed.). Washington, DC: American Psychiatric Association.

[12] American Psychiatric Association (1994). Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (4th ed.). Washington, DC: American Psychiatric Press, Inc.

[13] Andreasen NC (2000). Schizophrenia: the fundamental questions. Brain Res Brain Res Rev 31(2-3):106–112.

[14] Anseloni VCZ, He F, Novikova SI, Turnbach Robbins M, Lidow IA, Ennis M, Lidow MS (2005). Alterations in stress-associated behaviors and neurochemical markers in adult rats after neonatal short-lasting local inflammatory insult. Neuroscience 131(3):635–645.

[15] Antonarakis SE (1998). Recommendations for a nomenclature system for human gene mutations. Nomenclature Working Group. Hum Mutat 11(1):1–3.

[16] Arinami T, Ohtsuki T, Takase K, Shimizu H, Yoshikawa T, Horigome H, Nakayama J, et al (2001). Screening for 22q11 deletions in a schizophrenia population. Schizophr Res 52(3):167–170.

[17] Arolt V, Lencer R, Nolte A, Muller-Myhsok B, Purmann S, Schurmann M, Leutelt J, et al (1996). Eye tracking dysfunction is a putative phenotypic susceptibility marker of schizophrenia and maps to a locus on chromosome 6p in families with multiple occurrence of the disease. Am J Med Genet 67(6):564–579.

[18] Arseneault L, Cannon M, Poulton R, Murray R, Caspi A, Moffitt TE (2002). Cannabis use in adolescence and risk for adult psychosis: longitudinal prospective study. BMJ 325(7374):1212–1213.

[19] Auso E, Lavado-Autric R, Cuevas E, Del Rey FE, Morreale De Escobar G, Berbel P (2004). A moderate and transient deficiency of maternal thyroid function at the beginning of fetal neocorticogenesis alters neuronal migration. Endocrinology 145(9):4037–4047.

[20] Azevedo M, Valente J, Macedo A, Dourado A, Coelho I, Pato M, Pato C (1993). Versão Portuguesa da "Entrevista Diagnóstica para Estudos Genéticos". Psiquiatria Clínica 14(4):213–17.

[21] Azevedo MH, Soares MJ, Coelho I, Dourado A, Valente J, Macedo A, Pato M, et al (1999). Using consensus OPCRIT diagnoses. An efficient procedure for best-estimate lifetime diagnoses. Br J Psychiatry 175:154–157.

[22] Badner JA, Gershon ES (2002). Meta-analysis of whole-genome linkage scans of bipolar disorder and schizophrenia. Mol Psychiatry 7(4):405–411.

[23] Balmer JE, Blomhoff R (2002). Gene expression regulation by retinoic acid. J Lipid Res 43(11):1773–1808.

[24] Bannon MJ, Pruetz B, Manning-Bog AB, Whitty CJ, Michelhaugh SK, Sacchetti P, Granneman JG, et al (2002). Decreased expression of the transcription factor NURR1 in dopamine neurons of cocaine abusers. Proc Natl Acad Sci U S A 99(9):6382–6385.

[25] Bassett AS, Hodgkinson K, Chow EW, Correia S, Scutt LE, Weksberg R (1998). 22q11 deletion syndrome in adults with schizophrenia. Am J Med Genet 81(4):328–337.

[26] Baumgartner A, Pietzcker A, Gaebel W (2000). The hypothalamic-pituitary-thyroid axis in patients with schizophrenia. Schizophr Res 44(3):233–243.

[27] Bäckman C, You ZB, Perlmann T, Hoffer BJ (2003). Elevated locomotor activity without altered striatal dopamine contents in Nurr1 heterozygous mice after acute exposure to methamphetamine. Behav Brain Res 143(1):95–100.

[28] Beckmann H (1999). Developmental malformations in cerebral structures of schizophrenic patients. Eur Arch Psychiatry Clin Neurosci 249 Suppl 4:44–47.

[29] Benvenga S, Cahnmann HJ, Robbins J (1993). Characterization of thyroid hormone binding to apolipoprotein-E: localization of the binding site in the exon 3-coded domain. Endocrinology 133(3):1300–1305.

[30] Berlim MT, Mattevi BS, Belmonte-de Abreu P, Crow TJ (2003). The etiology of schizophrenia and the origin of language: overview of a theory. Compr Psychiatry 44(1):7–14.

[31] Beuckmann CT, Aoyagi M, Okazaki I, Hiroike T, Toh H, Hayaishi O, Urade Y (1999). Binding of biliverdin, bilirubin, and thyroid hormones to lipocalin-type prostaglandin D synthase. Biochemistry 38(25):8006–8013.

[32] Blake CC, Geisow MJ, Oatley SJ, Rerat B, Rerat C (1978). Structure of prealbumin: secondary, tertiary and quaternary interactions determined by Fourier refinement at 1.8 A. J Mol Biol 121(3):339–356.

[33] Blödorn B, Mäder M, Urade Y, Hayaishi O, Felgenhauer K, Brück W (1996). Choroid plexus: the major site of mRNA expression for the beta-trace protein (prostaglandin D synthase) in human brain. Neurosci Lett 209(2):117–120.

[34] Bock E (1978). Immunoglobulins, prealbumin, transferrin, albumin, and alpha2-macroglobulin in cerebrospinal fluid and serum in schizophrenic patients. Birth Defects Orig Artic Ser 14(5):283–295.

[35] Bosetti F, Weerasinghe GR, Rosenberger TA, Rapoport SI (2003). Valproic acid down-regulates the conversion of arachidonic acid to eicosanoids via cyclooxygenase-1 and -2 in rat brain. J Neurochem 85(3):690–696.

[36] Botstein D, Risch N (2003). Discovering genotypes underlying human phenotypes: past successes for mendelian disease, future approaches for complex disease. Nat Genet 33 Suppl:228–237.

[37] Breier A (2003). Diagnostic classification of the psychoses: historical context and implications for neurobiology. In D. S. Charney and E. J. Nestler (Eds.), *Neurobiology of Mental Illness* (2nd ed.)., pp. 237–246. New York, NY: Oxford University Press.

[38] Breier A, Su TP, Saunders R, Carson RE, Kolachana BS, de Bartolomeis A, Weinberger DR, et al (1997). Schizophrenia is associated with elevated

amphetamine-induced synaptic dopamine concentrations: evidence from a novel positron emission tomography method. Proc Natl Acad Sci U S A 94(6):2569–2574.

[39] Broadbelt K, Byne W, Jones LB (2002). Evidence for a decrease in basilar dendrites of pyramidal cells in schizophrenic medial prefrontal cortex. Schizophr Res 58(1):75–81.

[40] Broadbelt K, Ramprasaud A, Jones LB (2006). Evidence of altered neurogranin immunoreactivity in areas 9 and 32 of schizophrenic prefrontal cortex. Schizophrenia Research 87(1-3):6–14.

[41] Brown AS, Begg MD, Gravenstein S, Schaefer CA, Wyatt RJ, Bresnahan M, Babulas VP, et al (2004). Serologic evidence of prenatal influenza in the etiology of schizophrenia. Arch Gen Psychiatry 61(8):774–780.

[42] Brown AS, Susser ES (2002). In utero infection and adult schizophrenia. Ment Retard Dev Disabil Res Rev 8(1):51–57.

[43] Brunello N, Masotto C, Steardo L, Markstein R, Racagni G (1995). New insights into the biology of schizophrenia through the mechanism of action of clozapine. Neuropsychopharmacology 13(3):177–213.

[44] Buervenich S, Carmine A, Arvidsson M, Xiang F, Zhang Z, Sydow O, Jönsson EG, et al (2000). NURR1 mutations in cases of schizophrenia and manic-depressive disorder. Am J Med Genet 96(6):808–813.

[45] Buka SL, Tsuang MT, Torrey EF, Klebanoff MA, Wagner RL, Yolken RH (2001). Maternal cytokine levels during pregnancy and adult psychosis. Brain Behav Immun 15(4):411–420.

[46] Burghes AH, Vaessin HE, de La Chapelle A (2001). Genetics. The land between Mendelian and multifactorial inheritance. Science 293(5538):2213–2214.

[47] Cannon M, Jones PB, Murray RM (2002). Obstetric complications and schizophrenia: historical and meta-analytic review. Am J Psychiatry 159(7):1080–1092.

[48] Cannon TD, Zorrilla LE, Shtasel D, Gur RE, Gur RC, Marco EJ, Moberg P, et al (1994). Neuropsychological functioning in siblings discordant for schizophrenia and healthy volunteers. Arch Gen Psychiatry 51(8):651–661.

[49] Cantor-Graae E, Selten JP (2005). Schizophrenia and migration: a meta-analysis and review. Am J Psychiatry 162(1):12–24.

[50] Cardno AG, Gottesman II (2000). Twin studies of schizophrenia: from bow-and-arrow concordances to star wars Mx and functional genomics. Am J Med Genet 97(1):12–17.

[51] Carlsson A (1988). The current status of the dopamine hypothesis of schizophrenia. Neuropsychopharmacology 1(3):179–186.

[52] Carmine A, Buervenich S, Galter D, Jönsson EG, Sedvall GC, Farde L, Gustavsson JP, et al (2003). NURR1 promoter polymorphisms: Parkinson's disease, schizophrenia, and personality traits. Am J Med Genet B Neuropsychiatr Genet 120(1):51–57.

[53] Carrasco N (2000). Thyroid hormone synthesis. In L. E. Braverman and R. D. Utiger (Eds.), *In The thyroid. A fundamental and clinical text*, pp. 52–90. Philadelphia, NY: Lippincott Williams & Wilkins.

[54] Carro E, Trejo JL, Gerber A, Loetscher H, Torrado J, Metzger F, Torres-Aleman I (2006). Therapeutic actions of insulin-like growth factor I on APP/PS2 mice with severe brain amyloidosis. Neurobiol Aging 27(9):1250–1257.

[55] Castillo SO, Baffi JS, Palkovits M, Goldstein DS, Kopin IJ, Witta J, Magnuson MA, et al (1998). Dopamine biosynthesis is selectively abolished in substantia nigra/ventral tegmental area but not in hypothalamic neurons in mice with targeted disruption of the Nurr1 gene. Mol Cell Neurosci 11(1-2):36–46.

[56] Chakravarti A (1999). Population genetics–making sense out of sequence. Nat Genet 21(1 Suppl):56–60.

[57] Chambon P (1996). A decade of molecular biology of retinoic acid receptors. FASEB J 10(9):940–954.

[58] Chan S, Kilby MD (2000). Thyroid hormone and central nervous system development. J Endocrinol 165(1):1–8.

[59] Chen ML, Chen CH (2007). Comparative proteome analysis revealed up-regulation of transthyretin in rat brain under chronic clozapine treatment. J Psychiatr Res 41(1-2):63–68.

[60] Chen YH, Tsai MT, Shaw CK, Chen CH (2001). Mutation analysis of the human NR4A2 gene, an essential gene for midbrain dopaminergic neurogenesis, in schizophrenic patients. Am J Med Genet 105(8):753–757.

[61] Chiang MY, Misner D, Kempermann G, Schikorski T, Giguere V, Sucov HM, Gage FH, et al (1998). An essential role for retinoid receptors RARbeta and RXRgamma in long-term potentiation and depression. Neuron 21(6):1353–1361.

[62] Chopra IJ, Sabatino L (2000). Nature and sources of circulating thyroid hormone. In L. E. Braverman and R. D. Utiger (Eds.), *In The thyroid. A fundamental and clinical text*, pp. 121–135. Philadelphia, NY: Lippincott Williams & Wilkins.

[63] Chowdari KV, Mirnics K, Semwal P, Wood J, Lawrence E, Bhatia T, Deshpande SN, et al (2002). Association and linkage analyses of RGS4 polymorphisms in schizophrenia. Hum Mol Genet 11(12):1373–1380.

[64] Chumakov I, Blumenfeld M, Guerassimenko O, Cavarec L, Palicio M, Abderrahim H, Bougueleret L, et al (2002). Genetic and physiological data implicating the new human gene G72 and the gene for D-amino acid oxidase in schizophrenia. Proc Natl Acad Sci U S A 99(21):13675–13680.

[65] Chung C, Tallerico T, Seeman P (2003). Schizophrenia hippocampus has elevated expression of chondrex glycoprotein gene. Synapse 50(1):29–34.

[66] Citver AS, Shields AM, Ciaccia LM, Schulingkamp RJ, Raffa RB (2002). Indirect modulation of dopamine D2 receptors as potential pharmacotherapy for schizophrenia: III. Retinoids. J Clin Pharm Ther 27(3):161–168.

[67] Clayton D (1999). A generalization of the transmission/disequilibrium test for uncertain-haplotype transmission. Am J Hum Genet 65(4):1170–1177.

[68] Dalman C, Allebeck P, Cullberg J, Grunewald C, Koster M (1999). Obstetric complications and the risk of schizophrenia: a longitudinal study of a national birth cohort. Arch Gen Psychiatry 56(3):234–240.

[69] Davidson M, Reichenberg A, Rabinowitz J, Weiser M, Kaplan Z, Mark M (1999). Behavioral and intellectual markers for schizophrenia in apparently healthy male adolescents. Am J Psychiatry 156(9):1328–1335.

[70] de Pee S, Dary O (2002). Biochemical indicators of vitamin A deficiency: serum retinol and serum retinol binding protein. J Nutr 132(9 Suppl):2895S–2901S.

[71] Dean B, Opeskin K, Pavey G, Hill C, Keks N (1997). Changes in protein kinase C and adenylate cyclase in the temporal lobe from subjects with schizophrenia. J Neural Transm 104(11-12):1371–1381.

[72] Delange F (2001). Iodine deficiency as a cause of brain damage. Postgrad Med J 77(906):217–220.

[73] Delange FM (2000). Endemic cretinism. In L. E. Braverman and R. D. Utiger (Eds.), *In The thyroid. A fundamental and clinical text*, pp. 743–754. Philadelphia, NY: Lippincott Williams & Wilkins.

[74] DeLisi LE, Boccio AM, Riordan H, Hoff AL, Dorfman A, McClelland J, Kushner M, et al (1991). Familial thyroid disease and delayed language development in first admission patients with schizophrenia. Psychiatry Res 38(1):39–50.

[75] DeLisi LE, Mesen A, Rodriguez C, Bertheau A, LaPrade B, Llach M, Riondet S, et al (2002). Genome-wide scan for linkage to schizophrenia in a Spanish-origin cohort from Costa Rica. Am J Med Genet 114(5):497–508.

[76] DeLisi LE, Smith AB, Razi K, Stewart J, Wang Z, Sandhu HK, Philibert RA (2000). Investigation of a candidate gene for schizophrenia on Xq13 previously associated with mental retardation and hypothyroidism. Am J Med Genet 96(3):398–403.

[77] Deltour L, Foglio MH, Duester G (1999). Impaired retinol utilization in Adh4 alcohol dehydrogenase mutant mice. Dev Genet 25(1):1–10.

[78] Delva L, Bastie JN, Rochette-Egly C, Kraiba R, Balitrand N, Despouy G, Chambon P, et al (1999). Physical and functional interactions between cellular retinoic acid binding protein II and the retinoic acid-dependent nuclear complex. Mol Cell Biol 19(10):7158–7167.

[79] Deng HW, Chen WM, Recker RR (2001). Population admixture: detection by Hardy-Weinberg test and its quantitative effects on linkage-disequilibrium methods for localizing genes underlying complex traits. Genetics 157(2):885–897.

[80] Dickson PW, Aldred AR, Marley PD, Bannister D, Schreiber G (1986). Rat choroid plexus specializes in the synthesis and the secretion of transthyretin (prealbumin). Regulation of transthyretin synthesis in choroid plexus is independent from that in liver. J Biol Chem 261(8):3475–3478.

[81] Dowling AL, Zoeller RT (2000). Thyroid hormone of maternal origin regulates the expression of RC3/neurogranin mRNA in the fetal rat brain. Brain Res Mol Brain Res 82(1-2):126–132.

[82] Duan J, Martinez M, Sanders AR, Hou C, Saitou N, Kitano T, Mowry BJ, et al (2004). Polymorphisms in the trace amine receptor 4 (TRAR4) gene on chromosome 6q23.2 are associated with susceptibility to schizophrenia. Am J Hum Genet 75(4):624–638.

[83] Duester G (2000). Families of retinoid dehydrogenases regulating vitamin A function: production of visual pigment and retinoic acid. Eur J Biochem 267(14):4315–4324.

[84] Duncan GE, Sheitman BB, Lieberman JA (1999). An integrated view of pathophysiological models of schizophrenia. Brain Res Brain Res Rev 29(2-3):250–264.

[85] Dunn JT, Delange F (2001). Damaged reproduction: the most important consequence of iodine deficiency. J Clin Endocrinol Metab 86(6):2360–2363.

[86] Dunn JT, Dunn AD (2000). Thyroglobulin: Chemistry, Biosynthesis, and Proteolysis. In L. E. Braverman and R. D. Utiger (Eds.), *In The thyroid. A fundamental and clinical text*, pp. 91–104. Philadelphia, NY: Lippincott Williams & Wilkins.

[87] Eastwood SL, Burnet PWJ, Harrison PJ (2005). Decreased hippocampal expression of the susceptibility gene PPP3CC and other calcineurin subunits in schizophrenia. Biol Psychiatry 57(7):702–710.

[88] Eells JB, Lipska BK, Yeung SK, Misler JA, Nikodem VM (2002). Nurr1-null heterozygous mice have reduced mesolimbic and mesocortical dopamine levels and increased stress-induced locomotor activity. Behav Brain Res 136(1):267–275.

[89] Eguchi Y, Eguchi N, Oda H, Seiki K, Kijima Y, Matsu-ura Y, Urade Y, et al (1997). Expression of lipocalin-type prostaglandin D synthase (beta-trace) in human heart

and its accumulation in the coronary circulation of angina patients. Proc Natl Acad Sci U S A 94(26):14689–14694.

[90] Ehrentheil OF (1957). Common medical disorders rarely found in psychotic patients. AMA Arch Neurol Psychiatry 77(2):178–186.

[91] Ellenbroek BA, Cools AR (2000). The long-term effects of maternal deprivation depend on the genetic background. Neuropsychopharmacology 23(1):99–106.

[92] Ellenbroek BA, van den Kroonenberg PT, Cools AR (1998). The effects of an early stressful life event on sensorimotor gating in adult rats. Schizophr Res 30(3):251–260.

[93] Enderlin V, Pallet V, Alfos S, Dargelos E, Jaffard R, Garcin H, Higueret P (1997). Age-related decreases in mRNA for brain nuclear receptors and target genes are reversed by retinoic acid treatment. Neurosci Lett 229(2):125–129.

[94] Eneqvist T, Lundberg E, Nilsson L, Abagyan R, Sauer-Eriksson AE (2003). The transthyretin-related protein family. Eur J Biochem 270(3):518–532.

[95] Eravci M, Pinna G, Meinhold H, Baumgartner A (2000). Effects of pharmacological and nonpharmacological treatments on thyroid hormone metabolism and concentrations in rat brain. Endocrinology 141(3):1027–1040.

[96] Erlenmeyer-Kimling L, Rock D, Roberts SA, Janal M, Kestenbaum C, Cornblatt B, Adamo UH, et al (2000). Attention, memory, and motor skills as childhood predictors of schizophrenia-related psychoses: the New York High-Risk Project. Am J Psychiatry 157(9):1416–1422.

[97] Ethier I, Beaudry G, St-Hilaire M, Milbrandt J, Rouillard C, Levesque D (2004). The transcription factor NGFI-B (Nur77) and retinoids play a critical role in acute neuroleptic-induced extrapyramidal effect and striatal neuropeptide gene expression. Neuropsychopharmacology 29(2):335–346.

[98] Faraone SV, Chen WJ, Goldstein JM, Tsuang MT (1994). Gender differences in age at onset of schizophrenia. Br J Psychiatry 164(5):625–629.

[99] Féart C, Mingaud F, Enderlin V, Husson M, Alfos S, Higueret P, Pallet V (2005). Differential effect of retinoic acid and triiodothyronine on the age-related hypoexpression of neurogranin in rat. Neurobiol Aging 26(5):729–738.

[100] Feng X, Jiang Y, Meltzer P, Yen PM (2000). Thyroid hormone regulation of hepatic genes in vivo detected by complementary DNA microarray. Mol Endocrinol 14(7):947–955.

[101] Fenton WS, Hibbeln J, Knable M (2000). Essential fatty acids, lipid membrane abnormalities, and the diagnosis and treatment of schizophrenia. Biol Psychiatry 47(1):8–21.

[102] Fisher DA, Oddie TH, Thompson CS (1971). Thyroidal thyronine and nonthyronine iodine secretion in euthyroid subjects. J Clin Endocrinol Metab 33(4):647–652.

[103] Fleck A (1989). Clinical and nutritional aspects of changes in acute-phase proteins during inflammation. Proc Nutr Soc 48(3):347–354.

[104] Forrest D, Reh TA, Rusch A (2002). Neurodevelopmental control by thyroid hormone receptors. Curr Opin Neurobiol 12(1):49–56.

[105] Franco MH, Weimer TA, Salzano FM (1982). Blood polymorphisms and racial admixture in two Brazilian populations. Am J Phys Anthropol 58(2):127–132.

[106] Freedman R, Adler LE, Leonard S (1999). Alternative phenotypes for the complex genetics of schizophrenia. Biol Psychiatry 45(5):551–558.

[107] Freedman R, Coon H, Myles-Worsley M, Orr-Urtreger A, Olincy A, Davis A, Polymeropoulos M, et al (1997). Linkage of a neurophysiological deficit in schizophrenia to a chromosome 15 locus. Proc Natl Acad Sci U S A 94(2):587–592.

[108] Gaertner TR, Putkey JA, Waxham MN (2004). RC3/Neurogranin and Ca2+/calmodulin-dependent protein kinase II produce opposing effects on the affinity of calmodulin for calcium. J Biol Chem 279(38):39374–39382.

[109] García-Fernández LF, Iñiguez MA, Rodríguez-Peña A, Muñoz A, Bernal J (1993). Brain-specific prostaglandin D2 synthetase mRNA is dependent on thyroid hormone during rat brain development. Biochem Biophys Res Commun 196(1):396–401.

[110] García-Fernández LF, Rausell E, Urade Y, Hayaishi O, Bernal J, Muñoz A (1997). Hypothyroidism alters the expression of prostaglandin D2 synthase/beta trace in specific areas of the developing rat brain. Eur J Neurosci 9(8):1566–1573.

[111] Garey LJ, Ong WY, Patel TS, Kanani M, Davis A, Mortimer AM, Barnes TR, et al (1998). Reduced dendritic spine density on cerebral cortical pyramidal neurons in schizophrenia. J Neurol Neurosurg Psychiatry 65(4):446–453.

[112] Geddes J, Freemantle N, Harrison P, Bebbington P (2000). Atypical antipsychotics in the treatment of schizophrenia: systematic overview and meta-regression analysis. BMJ 321(7273):1371–1376.

[113] Geddes JR, Lawrie SM (1995). Obstetric complications and schizophrenia: a meta-analysis. Br J Psychiatry 167(6):786–793.

[114] Gerber DJ, Hall D, Miyakawa T, Demars S, Gogos JA, Karayiorgou M, Tonegawa S (2003). Evidence for association of schizophrenia with genetic variation in the 8p21.3 gene, PPP3CC, encoding the calcineurin gamma subunit. Proc Natl Acad Sci U S A 100(15):8993–8998.

[115] Ghyselinck NB, Bavik C, Sapin V, Mark M, Bonnier D, Hindelang C, Dierich A, et al (1999). Cellular retinol-binding protein I is essential for vitamin A homeostasis. EMBO J 18(18):4903–4914.

[116] Gilbert ME (2004). Alterations in synaptic transmission and plasticity in area CA1 of adult hippocampus following developmental hypothyroidism. Brain Res Dev Brain Res 148(1):11–18.

[117] Gladkevich A, Kauffman HF, Korf J (2004). Lymphocytes as a neural probe: potential for studying psychiatric disorders. Prog Neuropsychopharmacol Biol Psychiatry 28(3):559–576.

[118] Glantz LA, Lewis DA (2000). Decreased dendritic spine density on prefrontal cortical pyramidal neurons in schizophrenia. Arch Gen Psychiatry 57(1):65–73.

[119] Glass CK, Rosenfeld MG (2000). The coregulator exchange in transcriptional functions of nuclear receptors. Genes Dev 14(2):121–141.

[120] Glinoer D, Delange F (2000). The potential repercussions of maternal, fetal, and neonatal hypothyroxinemia on the progeny. Thyroid 10(10):871–887.

[121] Goff DC, Coyle JT (2001). The emerging role of glutamate in the pathophysiology and treatment of schizophrenia. Am J Psychiatry 158(9):1367–1377.

[122] Gogos JA, Santha M, Takacs Z, Beck KD, Luine V, Lucas LR, Nadler JV, et al (1999). The gene encoding proline dehydrogenase modulates sensorimotor gating in mice. Nat Genet 21(4):434–439.

[123] Gold S, Arndt S, Nopoulos P, O'Leary DS, Andreasen NC (1999). Longitudinal study of cognitive function in first-episode and recent-onset schizophrenia. Am J Psychiatry 156(9):1342–1348.

[124] Goodman AB (1996). Congenital anomalies in relatives of schizophrenic probands may indicate a retinoid pathology. Schizophr Res 19(2-3):163–170.

[125] Goodman AB (1998). Three independent lines of evidence suggest retinoids as causal to schizophrenia. Proc Natl Acad Sci U S A 95(13):7240–7244.

[126] Gottesman II (1991). Schizophrenia genesis: the origins of madness. New York: W. H. Freeman and Company.

[127] Gottesman II, Gould TD (2003). The endophenotype concept in psychiatry: etymology and strategic intentions. Am J Psychiatry 160(4):636–645.

[128] Gottesman ME, Quadro L, Blaner WS (2001). Studies of vitamin A metabolism in mouse model systems. Bioessays 23(5):409–419.

[129] Graef IA, Wang F, Charron F, Chen L, Neilson J, Tessier-Lavigne M, Crabtree GR (2003). Neurotrophins and netrins require calcineurin/NFAT signaling to stimulate outgrowth of embryonic axons. Cell 113(5):657–670.

[130] Guadaño-Ferraz A, Escámez MJ, Morte B, Vargiu P, Bernal J (1997). Transcriptional induction of RC3/neurogranin by thyroid hormone: differential neuronal sensitivity is not correlated with thyroid hormone receptor distribution in the brain. Brain Res Mol Brain Res 49(1-2):37–44.

[131] Guidotti A, Dong E, Matsumoto K, Pinna G, Rasmusson AM, Costa E (2001). The socially-isolated mouse: a model to study the putative role of allopregnanolone and 5alpha-dihydroprogesterone in psychiatric disorders. Brain Res Brain Res Rev 37(1-3):110–115.

[132] Gurling HM, Kalsi G, Brynjolfson J, Sigmundsson T, Sherrington R, Mankoo BS, Read T, et al (2001). Genomewide genetic linkage analysis confirms the presence

of susceptibility loci for schizophrenia, on chromosomes 1q32.2, 5q33.2, and 8p21-22 and provides support for linkage to schizophrenia, on chromosomes 11q23.3-24 and 20q12.1-11.23. Am J Hum Genet 68(3):661–673.

[133] Haddow JE, Palomaki GE, Allan WC, Williams JR, Knight GJ, Gagnon J, O'Heir CE, et al (1999). Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. N Engl J Med 341(8):549–555.

[134] Hakak Y, Walker JR, Li C, Wong WH, Davis KL, Buxbaum JD, Haroutunian V, et al (2001). Genome-wide expression analysis reveals dysregulation of myelination-related genes in chronic schizophrenia. Proc Natl Acad Sci U S A 98(8):4746–4751.

[135] Hall FS, Wilkinson LS, Humby T, Robbins TW (1999). Maternal deprivation of neonatal rats produces enduring changes in dopamine function. Synapse 32(1):37–43.

[136] Hambrecht M, Hafner H (2000). Cannabis, vulnerability, and the onset of schizophrenia: an epidemiological perspective. Aust N Z J Psychiatry 34(3):468–475.

[137] Harrington MG, Aebersold R, Martin BM, Merril CR, Hood L (1993). Identification of a brain-specific human cerebrospinal fluid glycoprotein, beta-trace protein. Appl Theor Electrophor 3(5):229–234.

[138] Harrington MG, Fonteh AN, Biringer RG, R Hühmer AF, Cowan RP (2006). Prostaglandin D synthase isoforms from cerebrospinal fluid vary with brain pathology. Dis Markers 22(1-2):73–81.

[139] Harrington MG, Merril CR, Torrey EF (1985). Differences in cerebrospinal fluid proteins between patients with schizophrenia and normal persons. Clin Chem 31(5):722–726.

[140] Harrison EH, Hussain MM (2001). Mechanisms involved in the intestinal digestion and absorption of dietary vitamin A. J Nutr 131(5):1405–1408.

[141] Harrison PJ (1999). The neuropathology of schizophrenia. A critical review of the data and their interpretation. Brain 122 (Pt 4):593–624.

[142] Harrison PJ, Owen MJ (2003). Genes for schizophrenia? Recent findings and their pathophysiological implications. Lancet 361(9355):417–419.

[143] Heaton R, Paulsen JS, McAdams LA, Kuck J, Zisook S, Braff D, Harris J, et al (1994). Neuropsychological deficits in schizophrenics. Relationship to age, chronicity, and dementia. Arch Gen Psychiatry 51(6):469–476.

[144] Hellemans KGC, Benge LC, Olmstead MC (2004). Adolescent enrichment partially reverses the social isolation syndrome. Brain Res Dev Brain Res 150(2):103–115.

[145] Hennah W, Varilo T, Kestila M, Paunio T, Arajarvi R, Haukka J, Parker A, et al (2003). Haplotype transmission analysis provides evidence of association for DISC1 to schizophrenia and suggests sex-dependent effects. Hum Mol Genet 12(23):3151–3159.

[146] Henquet C, Krabbendam L, Spauwen J, Kaplan C, Lieb R, Wittchen HU, van Os J (2005). Prospective cohort study of cannabis use, predisposition for psychosis, and psychotic symptoms in young people. BMJ 330(7481):11.

[147] Heresco-Levy U, Javitt DC, Ermilov M, Mordel C, Silipo G, Lichtenstein M (1999). Efficacy of high-dose glycine in the treatment of enduring negative symptoms of schizophrenia. Arch Gen Psychiatry 56(1):29–36.

[148] Higgs BW, Elashoff M, Richman S, Barci B (2006). An online database for brain disease research. BMC Genomics 7:70.

[149] Highley JR, DeLisi LE, Roberts N, Webb JA, Relja M, Razi K, Crow TJ (2003). Sex-dependent effects of schizophrenia: an MRI study of gyral folding, and cortical and white matter volume. Psychiatry Res 124(1):11–23.

[150] Hoff AL, Kremen WS, Wieneke MH, Lauriello J, Blankfeld HM, Faustman WO, Csernansky JG, et al (2001). Association of estrogen levels with neuropsychological performance in women with schizophrenia. Am J Psychiatry 158(7):1134–1139.

[151] Holson RR, Gazzara RA, Ferguson SA, Ali SF, Laborde JB, Adams J (1997). Gestational retinoic acid exposure: a sensitive period for effects on neonatal mortality and cerebellar development. Neurotoxicol Teratol 19(5):335–346.

[152] Holzman PS (1992). Behavioral markers of schizophrenia useful for genetic studies. J Psychiatr Res 26(4):427–445.

[153] Hoppin JA, Tolbert PE, Taylor JA, Schroeder JC, Holly EA (2002). Potential for selection bias with tumor tissue retrieval in molecular epidemiology studies. Ann Epidemiol 12(1):1–6.

[154] Howes OD, McDonald C, Cannon M, Arseneault L, Boydell J, Murray RM (2004). Pathways to schizophrenia: the impact of environmental factors. Int J Neuropsychopharmacol 7 Suppl 1:S7–S13.

[155] Huang JTJ, Leweke FM, Oxley D, Wang L, Harris N, Koethe D, Gerth CW, et al (2006). Disease biomarkers in cerebrospinal fluid of patients with first-onset psychosis. PLoS Med 3(11):e428.

[156] Huang KP, Huang FL, Chen HC (1993). Characterization of a 7.5-kDa protein kinase C substrate (RC3 protein, neurogranin) from rat brain. Arch Biochem Biophys 305(2):570–580.

[157] Huang KP, Huang FL, Jäger T, Li J, Reymann KG, Balschun D (2004). Neurogranin/RC3 enhances long-term potentiation and learning by promoting calcium-mediated signaling. J Neurosci 24(47):10660–10669.

[158] Huber TJ, Rollnik J, Wilhelms J, von zur Muhlen A, Emrich HM, Schneider U (2001). Estradiol levels in psychotic disorders. Psychoneuroendocrinology 26(1):27–35.

[159] Hudson CJ, Lin A, Cogan S, Cashman F, Warsh JJ (1997). The niacin challenge test: clinical manifestation of altered transmembrane signal transduction in schizophrenia? Biol Psychiatry 41(5):507–513.

[160] Hulshoff Pol HE, Hoek HW, Susser E, Brown AS, Dingemans A, Schnack HG, van Haren NE, et al (2000). Prenatal exposure to famine and brain morphology in schizophrenia. Am J Psychiatry 157(7):1170–1172.

[161] Husson M, Enderlin V, Alfos S, Féart C, Higueret P, Pallet V (2003). Triiodothyronine administration reverses vitamin A deficiency-related hypo-expression of retinoic acid and triiodothyronine nuclear receptors and of neurogranin in rat brain. Br J Nutr 90(1):191–198.

[162] Husum H, Termeer E, Mathe AA, Bolwig TG, Ellenbroek BA (2002). Early maternal deprivation alters hippocampal levels of neuropeptide Y and calcitonin-gene related peptide in adult rats. Neuropharmacology 42(6):798–806.

[163] Hutchinson G, Haasen C (2004). Migration and schizophrenia: the challenges for European psychiatry and implications for the future. Soc Psychiatry Psychiatr Epidemiol 39(5):350–357.

[164] Ichinose H, Ohye T, Suzuki T, Sumi-Ichinose C, Nomura T, Hagino Y, Nagatsu T (1999). Molecular cloning of the human Nurr1 gene: characterization of the human gene and cDNAs. Gene 230(2):233–239.

[165] Iñiguez MA, Rodríguez-Peña A, Ibarrola N, Aguilera M, Munoz A, Bernal J (1993). Thyroid hormone regulation of RC3, a brain-specific gene encoding a protein kinase-C substrate. Endocrinology 133(2):467–473.

[166] Imai C, Sugai T, Iritani S, Niizato K, Nakamura R, Makifuchi T, Kakita A, et al (2001). A quantitative study on the expression of synapsin II and N-ethylmaleimide-sensitive fusion protein in schizophrenic patients. Neurosci Lett 305(3):185–188.

[167] Ingraham LJ, Kety SS (2000). Adoption studies of schizophrenia. Am J Med Genet 97(1):18–22.

[168] Ishiguro H, Okubo Y, Ohtsuki T, Yamakawa-Kobayashi K, Arinami T (2002). Mutation analysis of the retinoid X receptor beta, nuclear-related receptor 1, and peroxisome proliferator-activated receptor alpha genes in schizophrenia and alcohol dependence: possible haplotype association of nuclear-related receptor 1 gene to alcohol dependence. Am J Med Genet 114(1):15–23.

[169] Ito M, Yuan CX, Malik S, Gu W, Fondell JD, Yamamura S, Fu ZY, et al (1999). Identity between TRAP and SMCC complexes indicates novel pathways for the function of nuclear receptors and diverse mammalian activators. Mol Cell 3(3):361–370.

[170] Iwayama-Shigeno Y, Yamada K, Toyota T, Shimizu H, Hattori E, Yoshitsugu K, Fujisawa T, et al (2003). Distribution of haplotypes derived from three common variants of the NR4A2 gene in Japanese patients with schizophrenia. Am J Med Genet B Neuropsychiatr Genet 118(1):20–24.

[171] Jablensky A, Sartorius N, Ernberg G, Anker M, Korten A, Cooper JE, Day R, et al (1992). Schizophrenia: manifestations, incidence and course in different cultures. A World Health Organization ten-country study. Psychol Med Monogr Suppl 20:1–97.

[172] Jacobson DR, Alves IL, Saraiva MJ, Thibodeau SN, Buxbaum JN (1995). Transthyretin Ser 6 gene frequency in individuals without amyloidosis. Hum Genet 95(3):308–312.

[173] Jow GM, Yang TT, Chen CL (2006). Leptin and cholesterol levels are low in major depressive disorder, but high in schizophrenia. J Affect Disord 90(1):21–27.

[174] Joyce JN, Shane A, Lexow N, Winokur A, Casanova MF, Kleinman JE (1993). Serotonin uptake sites and serotonin receptors are altered in the limbic system of schizophrenics. Neuropsychopharmacology 8(4):315–336.

[175] Kabeya K, Ishikawa K, Katakai K, Watanabe M, Ohe Y, Wakabayashi K, Mori M (1998). Prostaglandin-D-synthase (beta-trace protein) levels in rat cerebrospinal fluid. Neuroreport 9(5):915–919.

[176] Kamal TJ, Molitch ME (1992). Effects of calcium channel blockade with verapamil on the prolactin responses to TRH, L-dopa, and bromocriptine. Am J Med Sci 304(5):289–293.

[177] Kangave D (1992). More enlightenment on the essence of applying Fisher's Exact test when testing for statistical significance using small sample data presented in a 2 x 2 table. West Afr J Med 11(3):179–184.

[178] Karayiorgou M, Morris MA, Morrow B, Shprintzen RJ, Goldberg R, Borrow J, Gos A, et al (1995). Schizophrenia susceptibility associated with interstitial deletions of chromosome 22q11. Proc Natl Acad Sci U S A 92(17):7612–7616.

[179] Kasatkina EP, Samsonova LN, Ivakhnenko VN, Ibragimova GV, Ryabykh AV, Naumenko LL, Evdokimova YA (2006). Gestational hypothyroxinemia and cognitive function in offspring. Neurosci Behav Physiol 36(6):619–624.

[180] Kaufmann CA, Suarez B, Malaspina D, Pepple J, Svrakic D, Markel PD, Meyer J, et al (1998). NIMH Genetics Initiative Millenium Schizophrenia Consortium: linkage analysis of African-American pedigrees. Am J Med Genet 81(4):282–289.

[181] Kendler KS, Diehl SR (1993). The genetics of schizophrenia: a current, genetic-epidemiologic perspective. Schizophr Bull 19(2):261–285.

[182] Keshavan MS, Dick E, Mankowski I, Harenski K, Montrose DM, Diwadkar V, DeBellis M (2002). Decreased left amygdala and hippocampal volumes in young offspring at risk for schizophrenia. Schizophr Res 58(2-3):173–183.

[183] Kety SS, Wender PH, Jacobsen B, Ingraham LJ, Jansson L, Faber B, Kinney DK (1994). Mental illness in the biological and adoptive relatives of schizophrenic adoptees. Replication of the Copenhagen Study in the rest of Denmark. Arch Gen Psychiatry 51(6):442–455.

[184] Kim HS, Hong SJ, LeDoux MS, Kim KS (2001). Regulation of the tyrosine hydroxylase and dopamine beta-hydroxylase genes by the transcription factor AP-2. J Neurochem 76(1):280–294.

[185] Kim KS, Kim CH, Hwang DY, Seo H, Chung S, Hong SJ, Lim JK, et al (2003). Orphan nuclear receptor Nurr1 directly transactivates the promoter activity of the tyrosine hydroxylase gene in a cell-specific manner. J Neurochem 85(3):622–634.

[186] Kirov G, Georgieva L, Nikolov I, Zammit S, Jones G, Poriazova N, Tolev T, et al (2003). Association analysis of the HOPA12bp polymorphism in schizophrenia and manic depressive illness. Am J Med Genet B Neuropsychiatr Genet 118(1):16–19.

[187] Knable MB, Torrey EF, Webster MJ, Bartko JJ (2001). Multivariate analysis of prefrontal cortical data from the Stanley Foundation Neuropathology Consortium. Brain Res Bull 55(5):651–659.

[188] Kohrle J (1999). Local activation and inactivation of thyroid hormones: the deiodinase family. Mol Cell Endocrinol 151(1-2):103–119.

[189] Koibuchi N, Chin WW (2000). Thyroid hormone action and brain development. Trends Endocrinol Metab 11(4):123–128.

[190] Krebs-Thomson K, Giracello D, Solis A, Geyer MA (2001). Post-weaning handling attenuates isolation-rearing induced disruptions of prepulse inhibition in rats. Behav Brain Res 120(2):221–224.

[191] Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, Heninger GR, et al (1994). Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. Arch Gen Psychiatry 51(3):199–214.

[192] Kulkarni J, Riedel A, de Castella AR, Fitzgerald PB, Rolfe TJ, Taffe J, Burger H (2001). Estrogen - a potential treatment for schizophrenia. Schizophr Res 48(1):137–144.

[193] Lahti AC, Koffel B, LaPorte D, Tamminga CA (1995). Subanesthetic doses of ketamine stimulate psychosis in schizophrenia. Neuropsychopharmacology 13(1):9–19.

[194] LaMantia AS (1999). Forebrain induction, retinoic acid, and vulnerability to schizophrenia: insights from molecular and genetic analysis in developing mice. Biol Psychiatry 46(1):19–30.

[195] Lane MA, Bailey SJ (2005). Role of retinoid signalling in the adult brain. Prog Neurobiol 75(4):275–293.

[196] Langlois MC, Beaudry G, Zekki H, Rouillard C, Levesque D (2001). Impact of antipsychotic drug administration on the expression of nuclear receptors in the neocortex and striatum of the rat brain. Neuroscience 106(1):117–128.

[197] Larsen PD, DeLallo L (1989). Cerebrospinal fluid transthyretin in the neonate and blood-cerebrospinal fluid barrier permeability. Ann Neurol 25(6):628–630.

[198] Laruelle M, Kegeles LS, Abi-Dargham A (2003). Glutamate, dopamine, and schizophrenia: from pathophysiology to treatment. Ann N Y Acad Sci 1003:138–158.

[199] Law AJ, Lipska BK, Weickert CS, Hyde TM, Straub RE, Hashimoto R, Harrison PJ, et al (2006). Neuregulin 1 transcripts are differentially expressed in schizophrenia and regulated by 5' SNPs associated with the disease. Proc Natl Acad Sci U S A 103(17):6747–6752.

[200] Law SW, Conneely OM, DeMayo FJ, O'Malley BW (1992). Identification of a new brain-specific transcription factor, NURR1. Mol Endocrinol 6(12):2129–2135.

[201] Lawlor BA, Davis KL (1992). Does modulation of glutamatergic function represent a viable therapeutic strategy in Alzheimer's disease? Biol Psychiatry 31(4):337–350.

[202] Lawrie SM, Abukmeil SS (1998). Brain abnormality in schizophrenia. A systematic and quantitative review of volumetric magnetic resonance imaging studies. Br J Psychiatry 172:110–120.

[203] Lawrie SM, Whalley H, Kestelman JN, Abukmeil SS, Byrne M, Hodges A, Rimmington JE, et al (1999). Magnetic resonance imaging of brain in people at high risk of developing schizophrenia. Lancet 353(9146):30–33.

[204] Le W, Conneely OM, He Y, Jankovic J, Appel SH (1999). Reduced Nurr1 expression increases the vulnerability of mesencephalic dopamine neurons to MPTP-induced injury. J Neurochem 73(5):2218–2221.

[205] Le WD, Xu P, Jankovic J, Jiang H, Appel SH, Smith RG, Vassilatis DK (2003). Mutations in NR4A2 associated with familial Parkinson disease. Nat Genet 33(1):85–89.

[206] Leonard BE (1997). Fundamentals of Psychopharmacology (2nd ed.). England, UK: John Wiley & Sons Ltd.

[207] Leonard JL, Koehrle J (2000). Intracellular Pathways of Iodothyronine Metabolism. In L. E. Braverman and R. D. Utiger (Eds.), *In The thyroid. A fundamental and clinical text*, pp. 136–173. Philadelphia, NY: Lippincott Williams & Wilkins.

[208] Leung A, Chue P (2000). Sex differences in schizophrenia, a review of the literature. Acta Psychiatr Scand Suppl 401:3–38.

[209] Lewis CM, Levinson DF, Wise LH, DeLisi LE, Straub RE, Hovatta I, Williams NM, et al (2003). Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: Schizophrenia. Am J Hum Genet 73(1):34–48.

[210] Lewis DA, Lieberman JA (2000). Catching up on schizophrenia: natural history and neurobiology. Neuron 28(2):325–334.

[211] Li J, Pak JH, Huang FL, Huang KP (1999). N-methyl-D-aspartate induces neurogranin/RC3 oxidation in rat brain slices. J Biol Chem 274(3):1294–1300.

[212] Liu H, Heath SC, Sobin C, Roos JL, Galke BL, Blundell ML, Lenane M, et al (2002). Genetic variation at the 22q11 PRODH2/DGCR6 locus presents an unusual pattern and increases susceptibility to schizophrenia. Proc Natl Acad Sci U S A 99(6):3717–3722.

[213] Liu W, Breen G, Zhang J, Li S, Gu N, Feng G, Bai S, et al (2003). Association of APOE gene with schizophrenia in Chinese: a possible risk factor in times of malnutrition. Schizophr Res 62(3):225–230.

[214] Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN (2003). Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. Nat Genet 33(2):177–182.

[215] Lohnes D, Mark M, Mendelsohn C, Dolle P, Dierich A, Gorry P, Gansmuller A, et al (1994). Function of the retinoic acid receptors (RARs) during development (I). Craniofacial and skeletal abnormalities in RAR double mutants. Development 120(10):2723–2748.

[216] Lu HC, Eichele G, Thaller C (1997). Ligand-bound RXR can mediate retinoid signal transduction during embryogenesis. Development 124(1):195–203.

[217] Luo T, Wagner E, Crandall JE, Drager UC (2004). A retinoic-acid critical period in the early postnatal mouse brain. Biol Psychiatry 56(12):971–980.

[218] Ma T, Guo J, Wang F (1993). The epidemiology of iodine-deficiency diseases in China. Am J Clin Nutr 57(2 Suppl):264S–266S.

[219] Machon RA, Mednick SA, Huttunen MO (1997). Adult major affective disorder after prenatal exposure to an influenza epidemic. Arch Gen Psychiatry 54(4):322–328.

[220] Mackay TFC (2004). The genetic architecture of quantitative traits: lessons from Drosophila. Curr Opin Genet Dev 14(3):253–257.

[221] Maden M, Gale E, Zile M (1998). The role of vitamin A in the development of the central nervous system. J Nutr 128(2 Suppl):471S–475S.

[222] Malhotra AK, Adler CM, Kennison SD, Elman I, Pickar D, Breier A (1997). Clozapine blunts N-methyl-D-aspartate antagonist-induced psychosis: a study with ketamine. Biol Psychiatry 42(8):664–668.

[223] Marcelis M, van Os J, Sham P, Jones P, Gilvarry C, Cannon M, McKenzie K, et al (1998). Obstetric complications and familial morbid risk of psychiatric disorders. Am J Med Genet 81(1):29–36.

[224] Marchini J, Donnelly P, Cardon LR (2005). Genome-wide strategies for detecting multiple loci that influence complex diseases. Nat Genet 37(4):413–417.

[225] Marenco S, Weinberger DR (2000). The neurodevelopmental hypothesis of schizophrenia: following a trail of evidence from cradle to grave. Dev Psychopathol 12(3):501–527.

[226] Martin ER, Bass MP, Gilbert JR, Pericak-Vance MA, Hauser ER (2003). Genotype-based association test for general pedigrees: the genotype-PDT. Genet Epidemiol 25(3):203–213.

[227] Martínez de Arrieta C, Morte B, Coloma A, Bernal J (1999). The human RC3 gene homolog, NRGN contains a thyroid hormone-responsive element located in the first intron. Endocrinology 140(1):335–343.

[228] Martínez de Arrieta C, Perez Jurado L, Bernal J, Coloma A (1997). Structure, organization, and chromosomal mapping of the human neurogranin gene (NRGN). Genomics 41(2):243–249.

[229] Mason JW, Kennedy JL, Kosten TR, Giller ELJ (1989). Serum thyroxine levels in schizophrenic and affective disorder diagnostic subgroups. J Nerv Ment Dis 177(6):351–358.

[230] Matsumoto M, Weickert CS, Beltaifa S, Kolachana B, Chen J, Hyde TM, Herman MM, et al (2003). Catechol O-methyltransferase (COMT) mRNA expression in the dorsolateral prefrontal cortex of patients with schizophrenia. Neuropsychopharmacology 28(8):1521–1530.

[231] Matsumura H, Takahata R, Hayaishi O (1991). Inhibition of sleep in rats by inorganic selenium compounds, inhibitors of prostaglandin D synthase. Proc Natl Acad Sci U S A 88(20):9046–9050.

[232] Mattai AA, Tossell J, Greenstein DK, Addington A, Clasen LS, Gornick MC, Seal J, et al (2006). Sleep disturbances in childhood-onset schizophrenia. Schizophr Res 86(1-3):123–129.

[233] McCaffery P, Drager UC (2000). Regulation of retinoic acid signaling in the embryonic nervous system: a master differentiation factor. Cytokine Growth Factor Rev 11(3):233–249.

[234] McDonald C, Grech A, Toulopoulou T, Schulze K, Chapple B, Sham P, Walshe M, et al (2002). Brain volumes in familial and non-familial schizophrenic probands and their unaffected relatives. Am J Med Genet 114(6):616–625.

[235] McDonald C, Murray RM (2000). Early and late environmental risk factors for schizophrenia. Brain Res Brain Res Rev 31(2-3):130–137.

[236] McGrath JJ, Pemberton MR, Welham JL, Murray RM (1994). Schizophrenia and the influenza epidemics of 1954, 1957 and 1959: a southern hemisphere study. Schizophr Res 14(1):1–8.

[237] McGuffin P, Farmer A (2001). Polydiagnostic approaches to measuring and classifying psychopathology. Am J Med Genet 105(1):39–41.

[238] McGuffin P, Owen MJ, Farmer AE (1995). Genetic basis of schizophrenia. Lancet 346(8976):678–682.

[239] McGuffin P, Owen MJ, Gottesman II (2002). Psychiatric Genetics and Genomics. New York, NY: Oxford University Press.

[240] McNeil TF, Cantor-Graae E (2000). Minor physical anomalies and obstetric complications in schizophrenia. Aust N Z J Psychiatry 34 Suppl:65–73.

[241] Meltzer HY, Matsubara S, Lee JC (1989). Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2 and serotonin2 pKi values. J Pharmacol Exp Ther 251(1):238–246.

[242] Mendelsohn C, Lohnes D, Decimo D, Lufkin T, LeMeur M, Chambon P, Mark M (1994). Function of the retinoic acid receptors (RARs) during development (II). Multiple abnormalities at various stages of organogenesis in RAR double mutants. Development 120(10):2749–2771.

[243] Meyer U, Feldon J, Schedlowski M, Yee BK (2005). Towards an immunoprecipitated neurodevelopmental animal model of schizophrenia. Neurosci Biobehav Rev 29(6):913–947.

[244] Meyer U, Nyffeler M, Engler A, Urwyler A, Schedlowski M, Knuesel I, Yee BK, et al (2006a). The time of prenatal immune challenge determines the specificity of inflammation-mediated brain and behavioral pathology. J Neurosci 26(18):4752–4762.

[245] Meyer U, Schwendener S, Feldon J, Yee BK (2006b). Prenatal and postnatal maternal contributions in the infection model of schizophrenia. Exp Brain Res 173(2):243–257.

[246] Mic FA, Haselbeck RJ, Cuenca AE, Duester G (2002). Novel retinoic acid generating activities in the neural tube and heart identified by conditional rescue of Raldh2 null mutant mice. Development 129(9):2271–2282.

[247] Middleton FA, Mirnics K, Pierri JN, Lewis DA, Levitt P (2002). Gene expression profiling reveals alterations of specific metabolic pathways in schizophrenia. J Neurosci 22(7):2718–2729.

[248] Middleton FA, Pato CN, Gentile KL, McGann L, Brown AM, Trauzzi M, Diab H, et al (2005a). Gene expression analysis of peripheral blood leukocytes from discordant sib-pairs with schizophrenia and bipolar disorder reveals points of convergence between genetic and functional genomic approaches. Am J Med Genet B Neuropsychiatr Genet 136(1):12–25.

[249] Middleton FA, Peng L, Lewis DA, Levitt P, Mirnics K (2005b). Altered expression of 14-3-3 genes in the prefrontal cortex of subjects with schizophrenia. Neuropsychopharmacology 30(5):974–983.

[250] Miklos GLG, Maleszka R (2004). Microarray reality checks in the context of a complex disease. Nat Biotechnol 22(5):615–621.

[251] Mirnics K, Middleton FA, Lewis DA, Levitt P (2001a). Analysis of complex brain disorders with gene expression microarrays: schizophrenia as a disease of the synapse. Trends Neurosci 24(8):479–486.

[252] Mirnics K, Middleton FA, Marquez A, Lewis DA, Levitt P (2000). Molecular characterization of schizophrenia viewed by microarray analysis of gene expression in prefrontal cortex. Neuron 28(1):53–67.

[253] Mirnics K, Middleton FA, Stanwood GD, Lewis DA, Levitt P (2001b). Disease-specific changes in regulator of G-protein signaling 4 (RGS4) expression in schizophrenia. Mol Psychiatry 6(3):293–301.

[254] Miwa Y, Takiuchi S, Kamide K, Yoshii M, Horio T, Tanaka C, Banno M, et al (2004). Identification of gene polymorphism in lipocalin-type prostaglandin D synthase and its association with carotid atherosclerosis in Japanese hypertensive patients. Biochem Biophys Res Commun 322(2):428–433.

[255] Miyakawa T, Leiter LM, Gerber DJ, Gainetdinov RR, Sotnikova TD, Zeng H, Caron MG, et al (2003). Conditional calcineurin knockout mice exhibit multiple abnormal behaviors related to schizophrenia. Proc Natl Acad Sci U S A 100(15):8987–8992.

[256] Miyamoto Y, Yamada K, Noda Y, Mori H, Mishina M, Nabeshima T (2001). Hyperfunction of dopaminergic and serotonergic neuronal systems in mice lacking the NMDA receptor epsilon1 subunit. J Neurosci 21(2):750–757.

[257] Moghaddam B (2003). Bringing order to the glutamate chaos in schizophrenia. Neuron 40(5):881–884.

[258] Moghal N, Sternberg PW (2003). A component of the transcriptional mediator complex inhibits RAS-dependent vulval fate specification in C. elegans. Development 130(1):57–69.

[259] Mohn AR, Gainetdinov RR, Caron MG, Koller BH (1999). Mice with reduced NMDA receptor expression display behaviors related to schizophrenia. Cell 98(4):427–436.

[260] Moises HW, Yang L, Kristbjarnarson H, Wiese C, Byerley W, Macciardi F, Arolt V, et al (1995). An international two-stage genome-wide search for schizophrenia susceptibility genes. Nat Genet 11(3):321–324.

[261] Moldin SO (1997). The maddening hunt for madness genes. Nat Genet 17(2):127–129.

[262] Moldin SO, Gottesman II (1997). At issue: genes, experience, and chance in schizophrenia–positioning for the 21st century. Schizophr Bull 23(4):547–561.

[263] Molotkov A, Deltour L, Foglio MH, Cuenca AE, Duester G (2002a). Distinct retinoid metabolic functions for alcohol dehydrogenase genes Adh1 and Adh4 in protection against vitamin A toxicity or deficiency revealed in double null mutant mice. J Biol Chem 277(16):13804–13811.

[264] Molotkov A, Fan X, Deltour L, Foglio MH, Martras S, Farres J, Pares X, et al (2002b). Stimulation of retinoic acid production and growth by ubiquitously expressed alcohol dehydrogenase Adh3. Proc Natl Acad Sci U S A 99(8):5337–5342.

[265] Monaco HL, Rizzi M, Coda A (1995). Structure of a complex of two plasma proteins: transthyretin and retinol-binding protein. Science 268(5213):1039–1041.

[266] Mons N, Enderlin V, Jaffard R, Higueret P (2001). Selective age-related changes in the PKC-sensitive, calmodulin-binding protein, neurogranin, in the mouse brain. J Neurochem 79(4):859–867.

[267] Morreale de Escobar G (2003). Maternal hypothyroxinemia versus hypothyroidism and potential neurodevelopmental. Alterations of her offspring. Ann Endocrinol (Paris) 64(1):51–52.

[268] Morreale de Escobar G, Obregon MJ, Escobar del Rey F (2004). Role of thyroid hormone during early brain development. Eur J Endocrinol 151 Suppl 3:25–37.

[269] Morriss-Kay GM, Sokolova N (1996). Embryonic development and pattern formation. FASEB J 10(9):961–968.

[270] Morrow JD, Awad JA, Oates JA, Roberts LJn (1992). Identification of skin as a major site of prostaglandin D2 release following oral administration of niacin in humans. J Invest Dermatol 98(5):812–815.

[271] Morrow JD, Parsons WGr, Roberts LJn (1989). Release of markedly increased quantities of prostaglandin D2 in vivo in humans following the administration of nicotinic acid. Prostaglandins 38(2):263–274.

[272] Mothet JP, Parent AT, Wolosker H, Brady ROJ, Linden DJ, Ferris CD, Rogawski MA, et al (2000). D-serine is an endogenous ligand for the glycine site of the N-methyl-D-aspartate receptor. Proc Natl Acad Sci U S A 97(9):4926–4931.

[273] Mukai J, Liu H, Burt RA, Swor DE, Lai WS, Karayiorgou M, Gogos JA (2004). Evidence that the gene encoding ZDHHC8 contributes to the risk of schizophrenia. Nat Genet 36(7):725–731.

[274] Murphy KC, Jones LA, Owen MJ (1999). High rates of schizophrenia in adults with velo-cardio-facial syndrome. Arch Gen Psychiatry 56(10):940–945.

[275] Napoli JL (1999). Interactions of retinoid binding proteins and enzymes in retinoid metabolism. Biochim Biophys Acta 1440(2-3):139–162.

[276] Neuner-Jehle M, Denizot JP, Mallet J (1996). Neurogranin is locally concentrated in rat cortical and hippocampal neurons. Brain Res 733(1):149–154.

[277] Newcomer ME (1995). Retinoid-binding proteins: structural determinants important for function. FASEB J 9(2):229–239.

[278] Niederreither K, Subbarayan V, Dolle P, Chambon P (1999). Embryonic retinoic acid synthesis is essential for early mouse post-implantation development. Nat Genet 21(4):444–448.

[279] NIMH Genetics Workgroup (1999). Genetics and mental disorders. Biological Psychiatry 45:559–573.

[280] Nobel S, Abrahmsen L, Oppermann U (2001). Metabolic conversion as a prereceptor control mechanism for lipophilic hormones. Eur J Biochem 268(15):4113–4125.

[281] Novak G, Seeman P, Tallerico T (2000). Schizophrenia: elevated mRNA for calcium-calmodulin-dependent protein kinase IIbeta in frontal cortex. Brain Res Mol Brain Res 82(1-2):95–100.

[282] Noy N (2000). Retinoid-binding proteins: mediators of retinoid action. Biochem J 348 Pt 3:481–495.

[283] Nurnberger JIJ, Blehar MC, Kaufmann CA, York-Cooler C, Simpson SG, Harkavy-Friedman J, Severe JB, et al (1994). Diagnostic interview for genetic studies. Rationale, unique features, and training. NIMH Genetics Initiative. Arch Gen Psychiatry 51(11):849–859.

[284] O'Donnell P, Grace AA (1998). Dysfunctions in multiple interrelated systems as the neurobiological bases of schizophrenic symptom clusters. Schizophr Bull 24(2):267–283.

[285] Offterdinger M, Schneider SM, Huber H, Grunt TW (1999). Expression of c-erbB-4/HER4 is regulated in T47D breast carcinoma cells by retinoids and vitamin D3. Biochem Biophys Res Commun 258(3):559–564.

[286] Ordentlich P, Yan Y, Zhou S, Heyman RA (2003). Identification of the antineoplastic agent 6-mercaptopurine as an activator of the orphan nuclear hormone receptor Nurr1. J Biol Chem 278(27):24791–24799.

[287] Os Jv (2004). Does the urban environment cause psychosis? Br J Psychiatry 184:287–288.

[288] Othman SS, Abdul Kadir K, Hassan J, Hong GK, Singh BB, Raman N (1994). High prevalence of thyroid function test abnormalities in chronic schizophrenia. Aust N Z J Psychiatry 28(4):620–624.

[289] Ott SL, Roberts S, Rock D, Allen J, Erlenmeyer-Kimling L (2002). Positive and negative thought disorder and psychopathology in childhood among subjects with adulthood schizophrenia. Schizophr Res 58(2-3):231–239.

[290] Owen MJ, O'Donovan MC, Gottesman II (2002). Schizophrenia. In P. McGuffin, M. J. Owen, and I. I. Gottesman (Eds.), *Psychiatric Genetics and Genomics*, pp. 247–266. New York, NY: Oxford University Press.

[291] Pak JH, Huang FL, Li J, Balschun D, Reymann KG, Chiang C, Westphal H, et al (2000). Involvement of neurogranin in the modulation of calcium/calmodulin-dependent protein kinase II, synaptic plasticity, and spatial learning: a study with knockout mice. Proc Natl Acad Sci U S A 97(21):11232–11237.

[292] Palha JA (2002). Transthyretin as a thyroid hormone carrier: function revisited. Clin Chem Lab Med 40(12):1292–1300.

[293] Palha JA, Goodman AB (2006). Thyroid hormones and retinoids: a possible link between genes and environment in schizophrenia. Brain Res Brain Res Rev 51(1):61–71.

[294] Pantelis C, Velakoulis D, McGorry PD, Wood SJ, Suckling J, Phillips LJ, Yung AR, et al (2003). Neuroanatomical abnormalities before and after onset of psychosis: a cross-sectional and longitudinal MRI comparison. Lancet 361(9354):281–288.

[295] Perlmann T, Jansson L (1995). A novel pathway for vitamin A signaling mediated by RXR heterodimerization with NGFI-B and NURR1. Genes Dev 9(7):769–782.

[296] Philibert RA (2006). A meta-analysis of the association of the HOPA12bp polymorphism and schizophrenia. Psychiatr Genet 16(2):73–76.

[297] Philibert RA, Sandhu HK, Hutton AM, Wang Z, Arndt S, Andreasen NC, Crowe R, et al (2001). Population-based association analyses of the HOPA12bp polymorphism for schizophrenia and hypothyroidism. Am J Med Genet 105(1):130–134.

[298] Porterfield SP (1994). Vulnerability of the developing brain to thyroid abnormalities: environmental insults to the thyroid system. Environ Health Perspect 102 Suppl 2:125–130.

[299] Preisig M, Fenton BT, Matthey ML, Berney A, Ferrero F (1999). Diagnostic interview for genetic studies (DIGS): inter-rater and test-retest reliability of the French version. Eur Arch Psychiatry Clin Neurosci 249(4):174–179.

[300] Prichard L, Deloulme JC, Storm DR (1999). Interactions between neurogranin and calmodulin in vivo. J Biol Chem 274(12):7689–7694.

[301] Pulver AE (2000). Search for schizophrenia susceptibility genes. Biol Psychiatry 47(3):221–230.

[302] Pulver AE, Nestadt G, Goldberg R, Shprintzen RJ, Lamacz M, Wolyniec PS, Morrow B, et al (1994). Psychotic illness in patients diagnosed with velo-cardio-facial syndrome and their relatives. J Nerv Ment Dis 182(8):476–478.

[303] Ramakers GM, Gerendasy DD, de Graan PN (1999). Substrate phosphorylation in the protein kinase Cgamma knockout mouse. J Biol Chem 274(4):1873–1874.

[304] Rapoport JL, Addington AM, Frangou S, Psych MRC (2005). The neurodevelopmental model of schizophrenia: update 2005. Mol Psychiatry 10(5):434–449.

[305] Riecher-Rössler A (2002). Oestrogen effects in schizophrenia and their potential therapeutic implications-review. Arch Womens Ment Health 5(3):111–118.

[306] Riley B, Kendler KS (2006). Molecular genetic studies of schizophrenia. Eur J Hum Genet 14(6):669–680.

[307] Rinieris P, Christodoulou GN, Souvatzoglou A, Koutras DA, Stefanis C (1980). Free-thyroxine index in schizophrenic patients before and after neuroleptic treatment. Neuropsychobiology 6(1):29–33.

[308] Rios DLS, Vargas AF, Torres MR, Zago AJ, Callegari-Jacques SM, Hutz MH (2003). Interaction between SREBP-1a and APOB polymorphisms influences total and low-density lipoprotein cholesterol levels in patients with coronary artery disease. Clin Genet 63(5):380–385.

[309] Rioux L, Arnold SE (2005). The expression of retinoic acid receptor alpha is increased in the granule cells of the dentate gyrus in schizophrenia. Psychiatry Res 133(1):13–21.

[310] Risch N (1990a). Genetic linkage and complex diseases, with special reference to psychiatric disorders. Genet Epidemiol 7(1):3–16.

[311] Risch N (1990b). Linkage strategies for genetically complex traits. I. Multilocus models. Am J Hum Genet 46(2):222–228.

[312] Risch N, Merikangas K (1996). The future of genetic studies of complex human diseases. Science 273(5281):1516–1517.

[313] Risch NJ (2000). Searching for genetic determinants in the new millennium. Nature 405(6788):847–856.

[314] Robbins J (2000). Thyroid hormone transport proteins and the physiology of hormone binding. In L. E. Braverman and R. D. Utiger (Eds.), *In The thyroid. A fundamental and clinical text*, pp. 105–120. Philadelphia, NY: Lippincott Williams & Wilkins.

[315] Roca RP, Blackman MR, Ackerley MB, Harman SM, Gregerman RI (1990). Thyroid hormone elevations during acute psychiatric illness: relationship to severity and distinction from hyperthyroidism. Endocr Res 16(4):415–447.

[316] Rockman MV, Wray GA (2002). Abundant raw material for cis-regulatory evolution in humans. Mol Biol Evol 19(11):1991–2004.

[317] Rodríguez-Peña A (1999). Oligodendrocyte development and thyroid hormone. J Neurobiol 40(4):497–512.

[318] Rodriguez-Sanchez P, Tejero-Diez P, Diez-Guerra FJ (1997). Glutamate stimulates neurogranin phosphorylation in cultured rat hippocampal neurons. Neurosci Lett 221(2-3):137–140.

[319] Rozen S, Skaletsky H (2000). Primer3 on the WWW for general users and for biologist programmers. Methods Mol Biol 132:365–386.

[320] Ruano D, Macedo A, Soares M, Valente J, Azevedo M, Hutz M, Gama C, et al (2006). Transthyretin: No association between serum levels or gene variants and schizophrenia. J Psychiatr Res.

[321] Ryan WG, Roddam RF, Grizzle WE (1994). Thyroid function screening in newly admitted psychiatric inpatients. Ann Clin Psychiatry 6(1):7–12.

[322] Sacchetti P, Dwornik H, Formstecher P, Rachez C, Lefebvre P (2002). Requirements for heterodimerization between the orphan nuclear receptor Nurr1 and retinoid X receptors. J Biol Chem 277(38):35088–35096.

[323] Sacchetti P, Mitchell TR, Granneman JG, Bannon MJ (2001). Nurr1 enhances transcription of the human dopamine transporter gene through a novel mechanism. J Neurochem 76(5):1565–1572.

[324] Saha S, Chant D, Welham J, McGrath J (2005). A systematic review of the prevalence of schizophrenia. PLoS Med 2(5):e141.

[325] Sakai Y, Meno C, Fujii H, Nishino J, Shiratori H, Saijoh Y, Rossant J, et al (2001). The retinoic acid-inactivating enzyme CYP26 is essential for establishing an uneven distribution of retinoic acid along the anterio-posterior axis within the mouse embryo. Genes Dev 15(2):213–225.

[326] Sakurada K, Ohshima-Sakurada M, Palmer TD, Gage FH (1999). Nurr1, an orphan nuclear receptor, is a transcriptional activator of endogenous tyrosine hydroxylase in neural progenitor cells derived from the adult brain. Development 126(18):4017–4026.

[327] Samad TA, Krezel W, Chambon P, Borrelli E (1997). Regulation of dopaminergic pathways by retinoids: activation of the D2 receptor promoter by members of the retinoic acid receptor-retinoid X receptor family. Proc Natl Acad Sci U S A 94(26):14349–14354.

[328] Saucedo-Cardenas O, Quintana-Hau JD, Le WD, Smidt MP, Cox JJ, De Mayo F, Burbach JP, et al (1998). Nurr1 is essential for the induction of the dopaminergic phenotype and the survival of ventral mesencephalic late dopaminergic precursor neurons. Proc Natl Acad Sci U S A 95(7):4013–4018.

[329] Scanlon MF, Toft AD (2000). Regulation of thyrotropin secretion. In L. E. Braverman and R. D. Utiger (Eds.), *In The thyroid. A fundamental and clinical text*, pp. 234–253. Philadelphia, NY: Lippincott Williams & Wilkins.

[330] Schmid RS, McGrath B, Berechid BE, Boyles B, Marchionni M, Sestan N, Anton ES (2003). Neuregulin 1-erbB2 signaling is required for the establishment of radial glia and their transformation into astrocytes in cerebral cortex. Proc Natl Acad Sci U S A 100(7):4251–4256.

[331] Schneider H, Salzano FM (1979). Gm allotypes and racial admixture in two Brazilian populations. Hum Genet 53(1):101–105.

[332] Schulman IG, Li C, Schwabe JW, Evans RM (1997). The phantom ligand effect: allosteric control of transcription by the retinoid X receptor. Genes Dev 11(3):299–308.

[333] Schulze K, McDonald C, Frangou S, Sham P, Grech A, Toulopoulou T, Walshe M, et al (2003). Hippocampal volume in familial and nonfamilial schizophrenic probands and their unaffected relatives. Biol Psychiatry 53(7):562–570.

[334] Schwarcz R, Rassoulpour A, Wu HQ, Medoff D, Tamminga CA, Roberts RC (2001). Increased cortical kynurenate content in schizophrenia. Biol Psychiatry 50(7):521–530.

[335] Seeman P, Lee T (1975). Antipsychotic drugs: direct correlation between clinical potency and presynaptic action on dopamine neurons. Science 188(4194):1217–1219.

[336] Selemon LD, Goldman-Rakic PS (1999). The reduced neuropil hypothesis: a circuit based model of schizophrenia. Biol Psychiatry 45(1):17–25.

[337] Serot JM, Christmann D, Dubost T, Couturier M (1997). Cerebrospinal fluid transthyretin: aging and late onset Alzheimer's disease. J Neurol Neurosurg Psychiatry 63(4):506–508.

[338] Shah SH, Vankar GK, Peet M, Ramchand CN (2000). Unmedicated schizophrenic patients have a reduced skin flush in response to topical niacin. Schizophr Res 43(2-3):163–164.

[339] Sharma T, Lancaster E, Lee D, Lewis S, Sigmundsson T, Takei N, Gurling H, et al (1998). Brain changes in schizophrenia. Volumetric MRI study of families multiply affected with schizophrenia–the Maudsley Family Study 5. Br J Psychiatry 173:132–138.

[340] Shenton ME, Dickey CC, Frumin M, McCarley RW (2001). A review of MRI findings in schizophrenia. Schizophr Res 49(1-2):1–52.

[341] Shi L, Fatemi SH, Sidwell RW, Patterson PH (2003). Maternal influenza infection causes marked behavioral and pharmacological changes in the offspring. J Neurosci 23(1):297–302.

[342] Shifman S, Bronstein M, Sternfeld M, Pisante-Shalom A, Lev-Lehman E, Weizman A, Reznik I, et al (2002). A highly significant association between a COMT haplotype and schizophrenia. Am J Hum Genet 71(6):1296–1302.

[343] Silverman EK, Palmer LJ (2000). Case-control association studies for the genetics of complex respiratory diseases. Am J Respir Cell Mol Biol 22(6):645–648.

[344] Sim K, Chong SA, Chan YH, Lum WM (2002). Thyroid dysfunction in chronic schizophrenia within a state psychiatric hospital. Ann Acad Med Singapore 31(5):641–644.

[345] Smesny S, Berger G, Rosburg T, Riemann S, Riehemann S, McGorry P, Sauer H (2003). Potential use of the topical niacin skin test in early psychosis – a combined approach using optical reflection spectroscopy and a descriptive rating scale. J Psychiatr Res 37(3):237–247.

[346] Smith JW, Evans AT, Costall B, Smythe JW (2002). Thyroid hormones, brain function and cognition: a brief review. Neurosci Biobehav Rev 26(1):45–60.

[347] Sobell JL, Mikesell MJ, McMurray CT (2002). Genetics and etiopathophysiology of schizophrenia. Mayo Clin Proc 77(10):1068–1082.

[348] Sockanathan S, Jessell TM (1998). Motor neuron-derived retinoid signaling specifies the subtype identity of spinal motor neurons. Cell 94(4):503–514.

[349] Sousa JC, Grandela C, Fernandez-Ruiz J, de Miguel R, de Sousa L, Magalhaes AI, Saraiva MJ, et al (2004). Transthyretin is involved in depression-like behaviour and exploratory activity. J Neurochem 88(5):1052–1058.

[350] St Clair D, Xu M, Wang P, Yu Y, Fang Y, Zhang F, Zheng X, et al (2005). Rates of adult schizophrenia following prenatal exposure to the Chinese famine of 1959-1961. JAMA 294(5):557–562.

[351] Stefansson H, Sigurdsson E, Steinthorsdottir V, Bjornsdottir S, Sigmundsson T, Ghosh S, Brynjolfsson J, et al (2002). Neuregulin 1 and susceptibility to schizophrenia. Am J Hum Genet 71(4):877–892.

[352] Stephens M, Smith NJ, Donnelly P (2001). A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 68(4):978–989.

[353] Straub RE, Jiang Y, MacLean CJ, Ma Y, Webb BT, Myakishev MV, Harris-Kerr C, et al (2002). Genetic variation in the 6p22.3 gene DTNBP1, the human ortholog of the mouse dysbindin gene, is associated with schizophrenia. Am J Hum Genet 71(2):337–348.

[354] Strawn JR, Ekhator NN, D'Souza BB, Geracioti TDJ (2004). Pituitary-thyroid state correlates with central dopaminergic and serotonergic activity in healthy humans. Neuropsychobiology 49(2):84–87.

[355] Suddath RL, Christison GW, Torrey EF, Casanova MF, Weinberger DR (1990). Anatomical abnormalities in the brains of monozygotic twins discordant for schizophrenia. N Engl J Med 322(12):789–794.

[356] Sui L, Gilbert ME (2003). Pre- and postnatal propylthiouracil-induced hypothyroidism impairs synaptic transmission and plasticity in area CA1 of the neonatal rat hippocampus. Endocrinology 144(9):4195–4203.

[357] Sullivan GM, Hatterer JA, Herbert J, Chen X, Roose SP, Attia E, Mann JJ, et al (1999). Low levels of transthyretin in the CSF of depressed patients. Am J Psychiatry 156(5):710–715.

[358] Susser E, Neugebauer R, Hoek HW, Brown AS, Lin S, Labovitz D, Gorman JM (1996). Schizophrenia after prenatal famine. Further evidence. Arch Gen Psychiatry 53(1):25–31.

[359] Takahata R, Matsumura H, Kantha SS, Kubo E, Kawase K, Sakai T, Hayaishi O (1993). Intravenous administration of inorganic selenium compounds, inhibitors of prostaglandin D synthase, inhibits sleep in freely moving rats. Brain Res 623(1):65–71.

[360] Talbot K, Eidem WL, Tinsley CL, Benson MA, Thompson EW, Smith RJ, Hahn CG, et al (2004). Dysbindin-1 is reduced in intrinsic, glutamatergic terminals of the hippocampal formation in schizophrenia. J Clin Invest 113(9):1353–1363.

[361] Tamminga CA (1998). Schizophrenia and glutamatergic transmission. Crit Rev Neurobiol 12(1-2):21–36.

[362] Tanaka T, Urade Y, Kimura H, Eguchi N, Nishikawa A, Hayaishi O (1997). Lipocalin-type prostaglandin D synthase (beta-trace) is a newly recognized type of retinoid transporter. J Biol Chem 272(25):15789–15795.

[363] Tienari P, Wynne LC, Sorri A, Lahti I, Laksy K, Moring J, Naarala M, et al (2004). Genotype-environment interaction in schizophrenia-spectrum disorder. Long-term follow-up study of Finnish adoptees. Br J Psychiatry 184:216–222.

[364] Tkachev D, Mimmack ML, Ryan MM, Wayland M, Freeman T, Jones PB, Starkey M, et al (2003). Oligodendrocyte dysfunction in schizophrenia and bipolar disorder. Lancet 362(9386):798–805.

[365] Todorova VK, Elbein AD, Kyosseva SV (2003). Increased expression of c-Jun transcription factor in cerebellar vermis of patients with schizophrenia. Neuropsychopharmacology 28(8):1506–1514.

[366] Torii T, Kawarai T, Nakamura S, Kawakami H (1999). Organization of the human orphan nuclear receptor Nurr1 gene. Gene 230(2):225–232.

[367] Toyoda N, Nishikawa M, Mori Y, Yoshimura M, Masaki H, Gondou A, Yonemoto T, et al (1992). Identification of a 27-kilodalton protein with the properties of type I iodothyronine 5'-deiodinase in human thyroid gland. J Clin Endocrinol Metab 74(3):533–538.

[368] Tsai G, Coyle JT (2002). Glutamatergic mechanisms in schizophrenia. Annu Rev Pharmacol Toxicol 42:165–179.

[369] Tsai G, Yang P, Chung LC, Lange N, Coyle JT (1998). D-serine added to antipsychotics for the treatment of schizophrenia. Biol Psychiatry 44(11):1081–1089.

[370] Tseng KY, Roubert C, Do L, Rubinstein M, Kelly MA, Grandy DK, Low MJ, et al (2000). Selective increase of Nurr1 mRNA expression in mesencephalic dopaminergic neurons of D2 dopamine receptor-deficient mice. Brain Res Mol Brain Res 80(1):1–6.

[371] Tsuang MT, Stone WS, Faraone SV (1999). Schizophrenia: a review of genetic studies. Harv Rev Psychiatry 7(4):185–207.

[372] Tsuang MT, Stone WS, Faraone SV (2001). Genes, environment and schizophrenia. Br J Psychiatry Suppl 40:18–24.

[373] Tsuzuki T, Mita S, Maeda S, Araki S, Shimada K (1985). Structure of the human prealbumin gene. J Biol Chem 260(22):12224–12227.

[374] Urade Y, Eguchi N (2002). Lipocalin-type and hematopoietic prostaglandin D synthases as a novel example of functional convergence. Prostaglandins Other Lipid Mediat 68-69:375–382.

[375] Urade Y, Eguchi N, Hayaishi O (2006). Lipocalin-Type Prostaglandin D Synthase as an Enzymic Lipocalin. In B. Åkerström, N. Borregaard, D. R. Flower, and J.-P. Salier (Eds.), *Lipocalins*, pp. 99–109. Georgetown: Landes Bioscience.

[376] Urade Y, Fujimoto N, Hayaishi O (1985). Purification and characterization of rat brain prostaglandin D synthetase. J Biol Chem 260(23):12410–12415.

[377] Van den Buuse M, Garner B, Koch M (2003). Neurodevelopmental animal models of schizophrenia: effects on prepulse inhibition. Curr Mol Med 3(5):459–471.

[378] Vandenbrouck Y, Janvier B, Loriette C, Bereziat G, Mangeney-Andreani M (1994). The modulation of apolipoprotein E gene expression by 3,3'-5-triiodothyronine in HepG2 cells occurs at transcriptional and post-transcriptional levels. Eur J Biochem 224(2):463–471.

[379] Vara H, Munoz-Cuevas J, Colino A (2003). Age-dependent alterations of long-term synaptic plasticity in thyroid-deficient rats. Hippocampus 13(7):816–825.

[380] Vawter MP, Barrett T, Cheadle C, Sokolov BP, Wood WHr, Donovan DM, Webster M, et al (2001). Application of cDNA microarrays to examine gene expression differences in schizophrenia. Brain Res Bull 55(5):641–650.

[381] Verdoux H (2004). Perinatal risk factors for schizophrenia: how specific are they? Curr Psychiatry Rep 6(3):162–167.

[382] Vermot J, Gallego Llamas J, Fraulob V, Niederreither K, Chambon P, Dolle P (2005). Retinoic acid controls the bilateral symmetry of somite formation in the mouse embryo. Science 308(5721):563–566.

[383] Vermot J, Pourquie O (2005). Retinoic acid coordinates somitogenesis and left-right patterning in vertebrate embryos. Nature 435(7039):215–220.

[384] Vidal-Puig A, Moller DE (1994). Comparative sensitivity of alternative single-strand conformation polymorphism (SSCP) methods. Biotechniques 17(3):490–492.

[385] Vitti P, Delange F, Pinchera A, Zimmermann M, Dunn JT (2003). Europe is iodine deficient. Lancet 361(9364):1226.

[386] Waldo MC (1999). Co-distribution of sensory gating and impaired niacin flush response in the parents of schizophrenics. Schizophr Res 40(1):49–53.

[387] Wall JD, Pritchard JK (2003). Haplotype blocks and linkage disequilibrium in the human genome. Nat Rev Genet 4(8):587–597.

[388] Wan C, Yang Y, Li H, La Y, Zhu H, Jiang L, Chen Y, et al (2006). Dysregulation of retinoid transporters expression in body fluids of schizophrenia patients. J Proteome Res 5(11):3213–3216.

[389] Wang HY, Zhang FC, Gao JJ, Fan JB, Liu P, Zheng ZJ, Xi H, et al (2000). Apolipoprotein E is a genetic risk factor for fetal iodine deficiency disorder in China. Mol Psychiatry 5(4):363–368.

[390] Wang J, Harris M, Amos B, Li M, Wang X, Zhang J, Chen J (1997). A ten year review of the iodine deficiency disorders program of the People's Republic of China. J Public Health Policy 18(2):219–241.

[391] Wang Z, Benoit G, Liu J, Prasad S, Aarnisalo P, Liu X, Xu H, et al (2003). Structure and function of Nurr1 identifies a class of ligand-independent nuclear receptors. Nature 423(6939):555–560.

[392] Ward KE, Friedman L, Wise A, Schulz SC (1996). Meta-analysis of brain and cranial size in schizophrenia. Schizophr Res 22(3):197–213.

[393] Ward PE, Sutherland J, Glen EM, Glen AI (1998). Niacin skin flush in schizophrenia: a preliminary report. Schizophr Res 29(3):269–274.

[394] Watson JB, Sutcliffe JG, Fisher RS (1992). Localization of the protein kinase C phosphorylation/calmodulin-binding substrate RC3 in dendritic spines of neostriatal neurons. Proc Natl Acad Sci U S A 89(18):8581–8585.

[395] Weickert CS, Straub RE, McClintock BW, Matsumoto M, Hashimoto R, Hyde TM, Herman MM, et al (2004). Human dysbindin (DTNBP1) gene expression in normal brain and in schizophrenic prefrontal cortex and midbrain. Arch Gen Psychiatry 61(6):544–555.

[396] Weinberger DR (1999). Cell biology of the hippocampal formation in schizophrenia. Biol Psychiatry 45(4):395–402.

[397] Weinberger DR, Lipska BK (1995). Cortical maldevelopment, anti-psychotic drugs, and schizophrenia: a search for common ground. Schizophr Res 16(2):87–110.

[398] Weiss IC, Di Iorio L, Feldon J, Domeney AM (2000). Strain differences in the isolation-induced effects on prepulse inhibition of the acoustic startle response and on locomotor activity. Behav Neurosci 114(2):364–373.

[399] Weiss IC, Pryce CR, Jongen-Relo AL, Nanz-Bahr NI, Feldon J (2004). Effect of social isolation on stress-related behavioural and neuroendocrine state in the rat. Behav Brain Res 152(2):279–295.

[400] White DM, Mikol DD, Espinosa R, Weimer B, Le Beau MM, Stefansson K (1992). Structure and chromosomal localization of the human gene for a brain form of prostaglandin D2 synthase. J Biol Chem 267(32):23202–23208.

[401] White DM, Takeda T, DeGroot LJ, Stefansson K, Arnason BG (1997). Beta-trace gene expression is regulated by a core promoter and a distal thyroid hormone response element. J Biol Chem 272(22):14387–14393.

[402] White PF, Way WL, Trevor AJ (1982). Ketamine–its pharmacology and therapeutic uses. Anesthesiology 56(2):119–136.

[403] Whybrow PC, Bauer M (2000a). Behavioral and Psychiatric Aspects of Hypothyroidism. In L. E. Braverman and R. D. Utiger (Eds.), *In The thyroid. A fundamental and clinical text*, pp. 837–842. Philadelphia, NY: Lippincott Williams & Wilkins.

[404] Whybrow PC, Bauer M (2000b). Behavioral and Psychiatric Aspects of Thyrotoxicosis. In L. E. Braverman and R. D. Utiger (Eds.), *In The thyroid. A fundamental and clinical text*, pp. 673–678. Philadelphia, NY: Lippincott Williams & Wilkins.

[405] Wiersinga WM (2000). Nonthyroidal Illness. In L. E. Braverman and R. D. Utiger (Eds.), *In The thyroid. A fundamental and clinical text*, pp. 281–295. Philadelphia, NY: Lippincott Williams & Wilkins.

[406] Wietrzych M, Meziane H, Sutter A, Ghyselinck N, Chapman PF, Chambon P, Krezel W (2005). Working memory deficits in retinoid X receptor gamma-deficient mice. Learn Mem 12(3):318–326.

[407] Wigginton JE, Cutler DJ, Abecasis GR (2005). A note on exact tests of Hardy-Weinberg equilibrium. Am J Hum Genet 76(5):887–893.

[408] Williams NM, Rees MI, Holmans P, Norton N, Cardno AG, Jones LA, Murphy KC, et al (1999). A two-stage genome scan for schizophrenia susceptibility genes in 196 affected sibling pairs. Hum Mol Genet 8(9):1729–1739.

[409] Wong CT, Tsoi WF, Saha N (1996). Acute phase proteins in male Chinese schizophrenic patients in Singapore. Schizophr Res 22(2):165–171.

[410] World Health Organization (1994). International Classification of Diseases (ICD-10) (10th ed.). Geneva: World Health Organization.

[411] Wu C, Zeng Q, Blumer KJ, Muslin AJ (2000). RGS proteins inhibit Xwnt-8 signaling in Xenopus embryonic development. Development 127(13):2773–2784.

[412] Yamashima T, Sakuda K, Tohma Y, Yamashita J, Oda H, Irikura D, Eguchi N, et al (1997). Prostaglandin D synthase (beta-trace) in human arachnoid and meningioma cells: roles as a cell marker or in cerebrospinal fluid absorption, tumorigenesis, and calcification process. J Neurosci 17(7):2376–2382.

[413] Yang Y, Wan C, Li H, Zhu H, La Y, Xi Z, Chen Y, et al (2006). Altered levels of acute phase proteins in the plasma of patients with schizophrenia. Anal Chem 78(11):3571–3576.

[414] Yao JK, Leonard S, Reddy RD (2000). Membrane phospholipid abnormalities in postmortem brains from schizophrenic patients. Schizophr Res 42(1):7–17.

[415] Yazici K, Yazici AE, Taneli B (2002). Different neuroendocrine profiles of remitted and nonremitted schizophrenic patients. Prog Neuropsychopharmacol Biol Psychiatry 26(3):579–584.

[416] Yen PM (2001). Physiological and molecular basis of thyroid hormone action. Physiol Rev 81(3):1097–1142.

[417] Zeng H, Chattarji S, Barbarosie M, Rondi-Reig L, Philpot BD, Miyakawa T, Bear MF, et al (2001). Forebrain-specific calcineurin knockout selectively impairs bidirectional synaptic plasticity and working/episodic-like memory. Cell 107(5):617–629.

[418] Zetterström RH, Solomin L, Jansson L, Hoffer BJ, Olson L, Perlmann T (1997). Dopamine neuron agenesis in Nurr1-deficient mice. Science 276(5310):248–250.

[419] Zetterström RH, Williams R, Perlmann T, Olson L (1996). Cellular expression of the immediate early transcription factors Nurr1 and NGFI-B suggests a gene regulatory role in several brain regions including the nigrostriatal dopamine system. Brain Res Mol Brain Res 41(1-2):111–120.

[420] Zhang H, Emmons SW (2000). A C. elegans mediator protein confers regulatory selectivity on lineage-specific expression of a transcription factor gene. Genes Dev 14(17):2161–2172.

[421] Zubin J, Spring B (1977). Vulnerability–a new view of schizophrenia. J Abnorm Psychol 86(2):103–126.